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ROY WALDO MINER

6-MERCAPTOPURINE

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December 6, 1954

Editor

ROY WALDO MINER

## 6-MERCAPTOPURINE\*

Conference Co-Chairman: GEORGE H. HITCHINGS AND C. P. RHODS

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# THE BIOSYNTHESIS OF NUCLEIC ACIDS AS A BASIS FOR AN APPROACH TO CHEMOTHERAPY\*

By George Bosworth Brown

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As background for discussion of the possible modes through which 6-mercaptopurine may be exerting its actions, it seems desirable to review briefly the picture of the biosynthesis of the nucleic acids. In particular, the incorporation of exogenously supplied moieties which are components of the nucleic acids, and the few known metabolic fates of certain inhibitory analogs related to these compounds, are pertinent.

The nucleic acids are present inside of all cells, and only inside of cells. They are never, for instance, circulating in the blood, as are certain proteins. Much circumstantial evidence has associated one type of nucleic acid with the morphologic forms in the nucleus which are characterized as the chromosomes, the carriers of the hereditary characteristics. That type of nucleic acids, the deoxyribonucleic acids (DNA), is the only one which deserves the name nucleic acid in the sense originally implied by Miescher. There is a definite constancy of the amount and composition of the deoxyribonucleic acids in the nuclei of the cells of any given species. They are formed chiefly in association with cell division, and are thereafter comparatively, but not completely, metabolically inert.

The second major type, the ribonucleic acids (RNA), carry the generic name, not because of location, but because of similarities in chemical composition. In the case of this more abundant type of nucleic acids, the bulk are found in the particulate matter of the cytoplasm of the cell. They vary in composition from tissue to tissue, and their quantity and metabolic activity are greatly influenced by such factors as the nutritional state of the animal. The ribonucleic acids vary in metabolic activity with physiological conditions and play some dynamic, but not yet properly delineated, role in the moment-to-moment biochemical and synthetic activities of the cell.

The apparent roles that these substances play in growth and metabolic processes has led to optimism in believing that interference with nucleic acid synthesis or metabolism can alter growth characteristics, preferably, of course, those of neoplastic tissues or those of invasive agents, such as viruses. An essentially random collection of over 1000 pyrimidines and purines, the largest number of which have been furnished by Doctor Hitching's group, have been tested empirically in the Sloan-Kettering Chemotherapy Division, and about 6 per cent of these substances have shown at least some degree of effectiveness in inhibiting tumor growth. The hope that the neoplastic cell can be specifically inhibited by compounds of these types accordingly receives backing, not only on the theoretical ground of a crucial role of nucleic acids in directing growth, but also from the promising results of empirical testing, the most promising of which is the subject of this symposium.

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For several years, our group has been interested in the question of how organisms go about assembling the nucleic acids. We are motivated by the expectation that knowledge of the actual processes which can be involved in the biosynthesis of nucleic acids will help to orient the empirical search for chemotherapeutic agents. The same experimental techniques can also be utilized to investigate the mechanisms by which recognized antimetabolites may exert their inhibitory actions.

The nucleic acids are made up of a few relatively simple primary units. There are four or, in a few cases, five different nitrogenous heterocycles, each molecule of which is joined with one sugar and one phosphate moiety in a nucleotide. The two major types of nucleic acids are recognized by the kind of sugar found in them. In the last few years the ideas of polynucleotide structure have grown both more concrete and also more complex, and Levene's picture of a simple tetranucleotide has fallen into discard. FIGURE 1 represents only the minimum amount of material which is necessary to depict one example of each type of covalent linkage now suggested as being involved in the ribose polynucleotides. The complete macromolecule may have scores or hundreds of nucleotides. There one each of the nucleotides derived from the two pyrimidines, uracil and cytosine, and one each from the two purines, adenine and guanine, are shown. The major backbone of the polynucleotide is based upon diesterification of the phosphates to the third and fifth hydroxyls of adjacent ribose derivatives. There is evidence which suggests both of the logical possibilities for branching of the main chain. The 2'-deoxyribonucleic acids possess a quite similar structure. There is less evidence for branching of the polynucleotide chain and, of course, the absence of 2'-hydroxyls eliminates the possibility of any branching from that position. Within the polynucleotide, several purine nucleotides are found to be adjacent, and several pyrimidine nucleotides are also adjacent to one another, as is suggested here. The most striking example of a nucleic acid showing a peculiar compositional difference is that of the *T<sub>even</sub>* phages in which hydroxymethyl cytosine is found instead of cytosine. No peculiar compositional or chemical characteristics have yet been defined for the nucleic acids of tumors.

Our group has been investigating the way in which the living organism can go about assembling, or biosynthesizing, this type of structure, and we have directed our attention more specifically to the purine-containing units of the polynucleotide. Early nutritional studies showed that organisms are able to synthesize *de novo* all of their nucleic acids, and that none of the organic constituents of the nucleic acids is necessary in the diet. Most of the knowledge of the origin of nucleic acid components has come from the administration of isotopically labeled compounds, and the use of degradative procedures which permit analysis of individual moieties of the products.

By the latter methods, the principle sources of the individual atoms of the purine ring, as represented by excretory uric acid, were elucidated by Buchanan, Sonne, and Delluva.<sup>1, 2</sup> The purine ring is assembled from glycine, CO<sub>2</sub>, NH<sub>3</sub>, and one-carbon units such as formic acid. The studies of Greenberg<sup>3</sup> suggest that ribose and phosphate are attached to a smaller precursor before the syn-



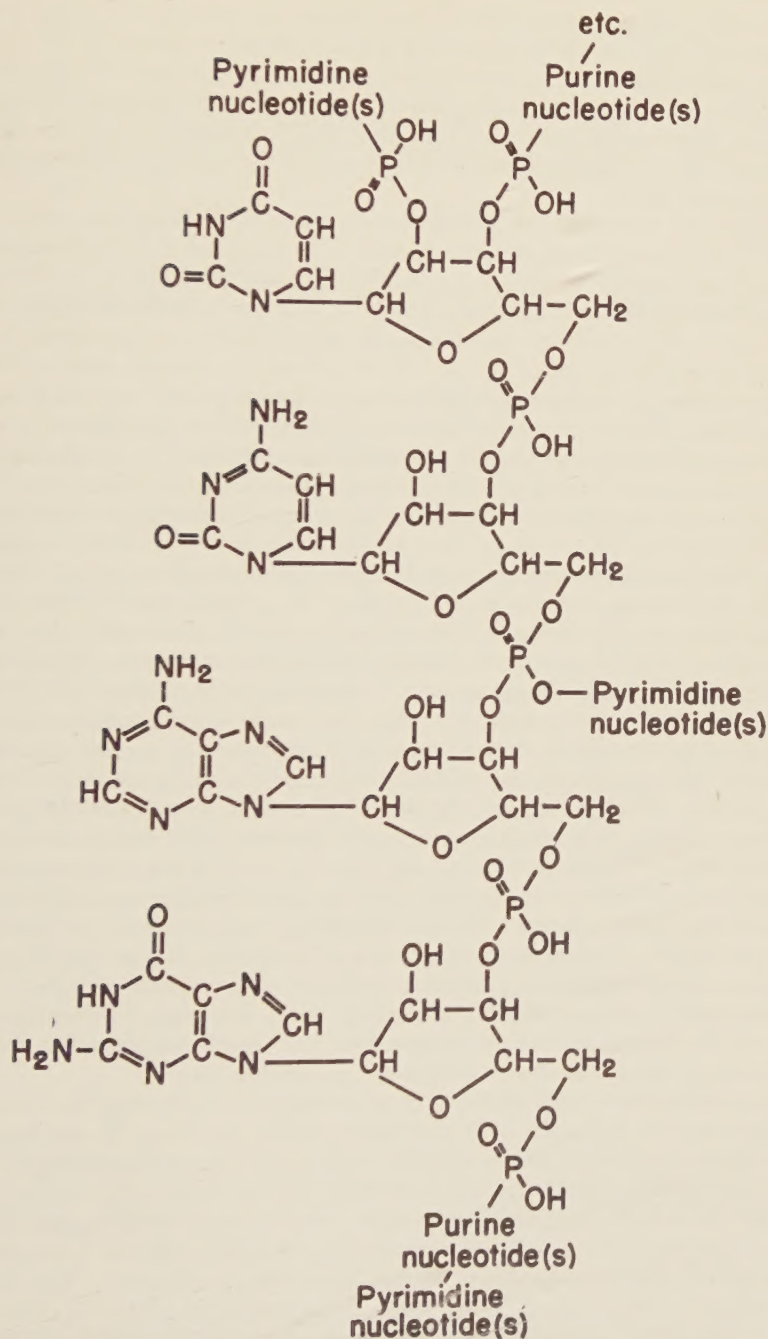


FIGURE 1. Fragment of a ribose polynucleotide.

thesis of the purine ring is completed, and there is no evidence that there is any formation of free purines in the course of biosyntheses in tissues.

Folic acid is involved in the metabolism of the one-carbon unit, including its incorporation into the purines and into the methyl group of thymine, and thus one place where the well known folic acid antagonists are presumed to interfere is at the level of the synthesis *de novo* of the nitrogenous heterocycles of the nucleic acids.

Despite the fact that free purines do not seem to be intermediates on the pathways of the synthesis *de novo*, it was to be expected that they might be utilized when available. However, in the early studies of Plentl and Schoenheimer<sup>4</sup> with N<sup>15</sup> labeled guanine, as well as with pyrimidines uracil and thymine, and a later study of cytosine by Bendich,<sup>5</sup> those were not found to be incorporated into the nucleic acids. In fact, those compounds, although they are components of nucleic acids, behave more like end-products of nucleic acid metabolism in that they enter chiefly into further catabolic reactions.

However, when isotopically labeled adenine was studied<sup>6</sup> it was found that its isotopes were incorporated extensively and specifically into the purine moieties of the polynucleotides of the rat. The isotopes appeared not only in the adenine of the nucleic acids but also in the guanine, and revealed that there could be extensive transformation of the one purine into the other. Degradation of the guanine which was derived from the administered adenine, later double-tracer experiments with both N<sup>15</sup> and with C<sup>14</sup>, and several other lines of evidence, have all indicated that this transformation is accomplished with the retention of the intact purine ring. Subsequent experiments with C<sup>14</sup> and C<sup>13</sup> labeled guanine have shown<sup>7, 8</sup> that there can be some small direct incorporation of guanine by the rat and, to a much larger extent, in other organisms. In several species, guanine can also be transformed into polynucleotide adenine.

In studies of the possible mechanism of this interconversion of the purines (FIGURE 2), the purines which were normally associated with mammalian purine metabolism were investigated, and none was found to be effectively converted into nucleic acid purines in the rat. However, one hypothetical candidate for a role as an intermediate in this transformation was a purine not known to occur in nature, 2,6-diaminopurine. It was found that, in the rat, it is converted into polynucleotide guanine about as readily as was adenine.<sup>9</sup> The isotopes were in the same relative positions as in the original diaminopurine, and the diaminopurine seemed to have been converted into a guanine derivative with an alteration of the substituent groups (FIGURE 3).

Since conversion of adenine into a guanine derivative (FIGURE 2) involves the replacement of a hydrogen in the 2-position with a functional group, one idea for a potentially useful antimetabolite was to attempt to interfere with that transformation by replacing the hydrogen with an unusual group such as methyl, chloro-, or other blocking groups. Among many such analogs tested, the 2-amino-derivative, or 2,6-diaminopurine, has been effective in interfering with growth or multiplication processes in a score of systems.<sup>10</sup> Any studies of the mechanism of its inhibitory action are complicated by the fact that it is also extensively metabolized into the usual nucleic acid purines. In a series of studies carried out with Balis, Elion, and Hitchings,<sup>11</sup> no evidence has



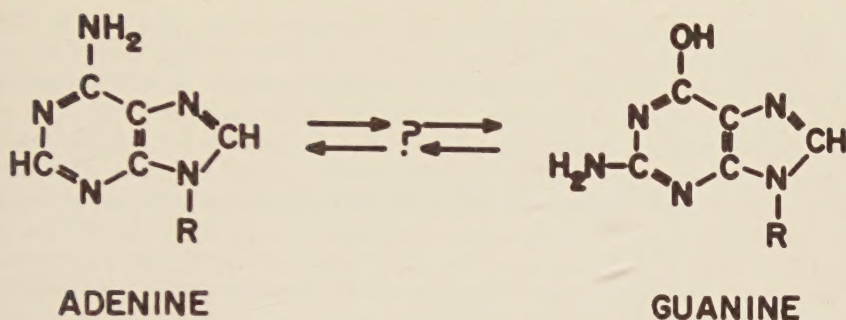


FIGURE 2.

been found that diaminopurine interferes directly with nucleic acid synthesis. Rather, it does appear that it competes with adenine in the synthesis of some other critical product which could be a coenzyme. Since adenine is also a component of many of the dinucleotide coenzymes, it must always be kept in mind that those vital units are possible sites of inhibitory action of purine analogs.

We have also studied several purine derivatives of the next higher levels of complexity: the ribosides and the ribotides. For the purposes of this discussion, the purine derivatives now known to function as precursors of polynucleotide purines in the rat are summarized in FIGURES 3 and 4. Here, the purines mentioned are on the left; the nucleosides, or ribosyl derivatives, in the center; then the nucleotides, or the phosphoribosyl derivatives, in the next group. Reference is made, in this diagram, only to the fate of the purine moieties. The darkest lines indicate extensive incorporation, the lighter solid lines indicate appreciable but less extensive incorporation, the broken lines indicate but trace incorporations. There are individual lines for each over-all conversion and no implication of a sequence of reactions is to be inferred. It can be seen that all of the adenine derivatives lead to polynucleotide adenine and also to polynucleotide guanine. However, the 2,6-diaminopurine and its riboside are almost exclusively converted into polynucleotide guanine. The fact that the guanine of guanylic acid is much more readily incorporated into polynucleotide than is free guanine, or its riboside, guanosine, indicates some unique metabolic property of the nucleotide.

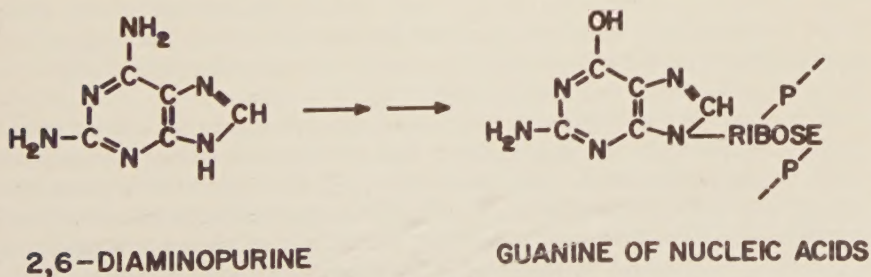


FIGURE 3.

This scheme represents the results with the Sherman rat, which is the only mammal for which we have this much data. In other species, mostly micro-organisms, there are pronounced differences in the pattern of interconversion of the purines. In some instances, guanine can be incorporated and converted into polynucleotide adenine, but adenine can be utilized for polynucleotide adenine synthesis only. In between, there are a number of species which can carry out the interconversion in both directions, sometimes with the emphasis on conversion in one direction, and sometimes in the other. In all species tested, the fate of diaminopurine has, in general, paralleled that of guanine. Even though free purines are not implicated as normal metabolic intermediates mechanisms for their efficient utilization exist in all species thus far tested. Those mechanisms are a fair target for chemotherapeutic attack, and the subject of this conference, 6-mercaptapurine, is one of those simple analogs of adenine in which the amino group is replaced by an unusual group.

One of the mechanisms by which analogs might act would be through their incorporation into the polynucleotides *in lieu* of the normal purine, and thus lead to a biologically ineffective PNA. In a few instances, minute traces of an analog have appeared to be present in nucleic acid preparations obtained from animals treated with the agent, but the distinction between true incorporation to the extent of one part in several thousand and contamination is an extremely difficult one. An analog of guanine for which antimetabolite types of activities have been found and which may operate in this manner is 8-azaguanine.<sup>12, 13</sup> Studies with that compound have furnished the one concrete demonstration of the incorporation of an abnormal purine analog into a polynucleotide. Matthews<sup>14</sup> has demonstrated its incorporation into tobacco mosaic virus (TMV) in plants and has isolated two isomeric 8-azaguanilyc acids from alkaline hydrolysates of that TMV. Perhaps a virus system furnishes a unique opportunity for such a demonstration. If the virus is a nonfunctional nucleoprotein in the cells which are synthesizing it, an appreciable quantity of the nucleic acid containing the abnormal analog may be accumulated, while if it were a functioning nucleoprotein a few molecules of the abnormal polynucleotide might lead to sufficient metabolic interference to stop further synthesis of it. Mandel,<sup>15</sup> however, has been able to demonstrate a small incorporation of 8-azaguanine into a nucleotide preparation from mouse PNA.

Among the pyrimidine bases of the nucleic acids, neither the uracil nor the cytosine moieties have been found serviceable as effective precursors in the rat. This finding is in contrast to the fact that certain purines can be very effectively incorporated. However, there are not only individual free purines and pyrimidines to consider, but there are also the corresponding nucleosides and nucleotides of each of these compounds.

FIGURES 4 and 5 constitute an overall flow sheet similar to the one for purine derivatives and again the heaviest lines indicate the most extensive incorporations. Information on the behavior of the pyrimidine derivatives came from studies with N<sup>15</sup>-labeled yeast nucleic acid,<sup>16</sup> the pyrimidine nucleosides,<sup>17</sup> and of their nucleotides,<sup>18, 19</sup> and showed that the ribosyl or phosphoribosyl derivatives of cytosine were incorporated to an extent comparable to that of adenine. The cytosine moiety of these derivatives was also converted into the



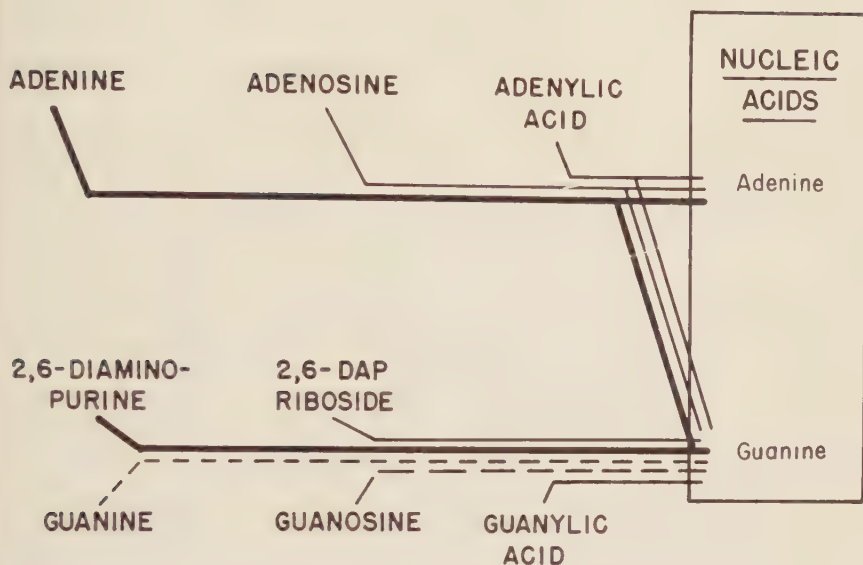


FIGURE 4. Derivatives which can function as precursors of the polynucleotide purines.

uracil component of the nucleic acids to a lesser extent, which is reminiscent of the interconversion of the purines which takes place. The corresponding derivatives of uracil are incorporated to a smaller extent, and there is a small conversion of them to cytosine. The deoxyribosyl derivatives of cytosine and thymine can likewise be utilized but, as indicated, only for the elaboration of the deoxyribonucleic acids.

In an experiment with uniformly labeled cytidine, Rose and Schweigert<sup>20</sup> found that the ribose as well as the cytosine moiety was incorporated and thus furnished a direct demonstration that the ribosyl linkage was maintained intact. Recent experiments by Roll and Weinfeld with cytidylic acids, labeled in pyrimidine, ribose, and phosphate moieties, have shown that the base and the ribose are equally incorporated, and also that the polynucleotide uridylic acid which was derived from it contained proportionate quantities of the isotopes in both the pyrimidine and the ribose. In other words, the nucleoside moiety appears to be metabolized as a unit during the transformation to the other pyrimidine derivative.

The phosphorus of the nucleotide was not specifically incorporated. Small amounts of  $P^{32}$  do arrive in the nucleic acids, but they are uniformly distributed among all of the phosphates of the nucleic acid, and the inorganic phosphate of the tissue was more extensively labeled than was the nucleic acid phosphorus. At least in the case of the 3' or 2'-isomers, accordingly, the nucleotide is not incorporated as a unit into the polynucleotides. The presence of the phosphate does not appreciably influence the fate of the nucleoside portion of the molecule, and that phosphate does not serve as a direct source of the phosphorus of the nucleotide unit of the nucleic acid.

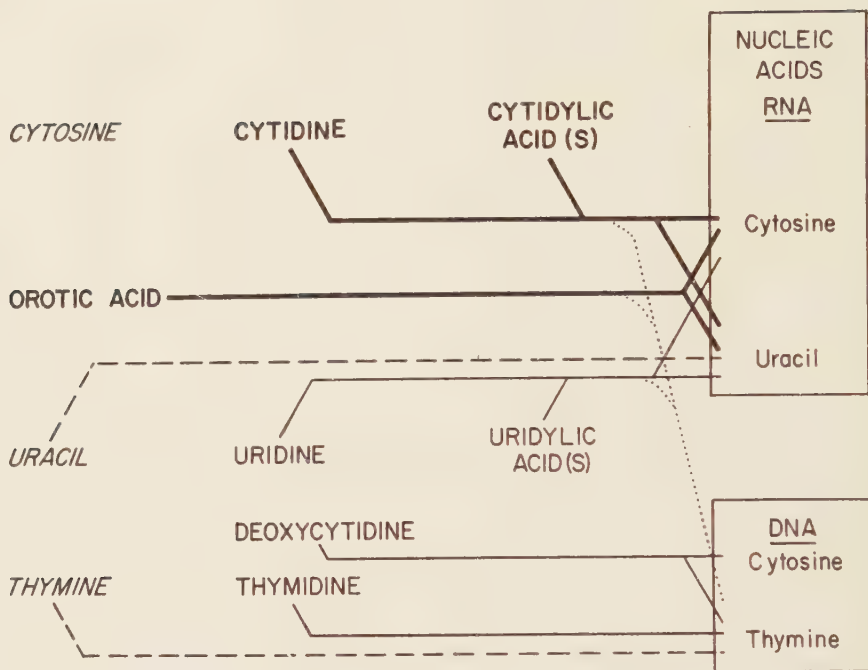


FIGURE 5. Derivatives which can function as precursors of the polynucleotide pyrimidines.

Labeled preparations of the nucleosides and nucleotides of several purines have been similarly studied. The purine moieties of all of the derivatives of adenine (adenosine and all of the adenylic acids) had been found to be utilized much less extensively than was the parent purine, in contrast to the situation with the pyrimidine derivatives where the ribosyl derivatives are extensively used but the free bases are not. In the case of adenylic acid, the 5'-isomer has been obtained and, in the rat, it is an even less effective precursor than is the nucleoside or the 2'- or 3'-phosphates. However, the presence in tissues of a large amount of acid soluble 5'-adenylic acid derivatives may have diluted the administered sample and may have strongly influenced the isotope dilution factors observed.

When an adenylic acid labeled with the three isotopes was studied,<sup>21</sup> the results were quite different from those obtained in the case of the pyrimidine nucleotides. Here again, the phosphorus was greatly diluted and was uniformly distributed throughout the nucleic acid, possibly via inorganic phosphate.<sup>22</sup> There was an extensive incorporation of the ribose into the adenylic acid of the polynucleotide, but this incorporation was only about 80 per cent as extensive as was the incorporation of the purine moiety. That 80 per cent could have been incorporated with the glycosidic linkage unit intact, but there was also some additional purine transferred into the polynucleotide without its accompanying ribose. The guanylic acid derived from the adenylic acid



received about equal amounts of isotopes in both the purine and ribose moieties, which indicates that, in the transformation of adenylic to PNA guanylic acid, a major pathway apparently involves retention of the intact nucleosidic linkage.

The situation regarding guanine and its derivatives is entirely different from that concerning adenine, and it also differs from the situation regarding the pyrimidine derivatives. In the rat, neither guanine nor its nucleoside was incorporated to an appreciable extent. However, in the case of either the 2'- or the 3'-nucleotides there was a quite extensive incorporation of the nitrogen and carbon of the guanine moiety into the guanine of the nucleic acids. Since guanylic acid was the only guanine-containing compound efficiently incorporated, it initially appeared logical to suggest<sup>19</sup> that the complete nucleotide might be the unit which was being incorporated into the nucleic acid.

Roll and Weinfeld<sup>21</sup> have now studied the triply labeled guanylic acids and have shown, however, that here, as in the case of all the other nucleotides, there is only a general nonspecific incorporation of its phosphorus. There was also but a relatively small incorporation of its ribose. At most, 20 per cent of the guanine incorporated could have been accompanied by ribose, and at least 80 per cent of the purine was incorporated independently of the rest of the molecule. Explanations of these observations will have to take into account the fact that free guanine is not incorporated extensively, and that the PNA guanine arising from adenylic acid was accompanied by an almost equal quantity of the ribose of that acid.

The virtue of the phosphoribosyl group of guanylic acid must lie in the fact that its presence permits the guanine moiety to be transferred, possibly via a transglycosidation type of mechanism, to some derivative which is a direct precursor of the polynucleotide. Whether that guanine moiety is transferred directly into the nucleic acid cannot yet be ascertained, but is a possibility to consider.

In brief: in the mammal, certain exogenously supplied free purines may be utilized for polynucleotide biosynthesis, but their nucleosides or nucleotides are generally less effectively utilized. Transfer of the purine moieties to a new glycosyl moiety or, to coin a word for emphasis, *transpurination* is involved to at least some extent in the course of the anabolism of these purine derivatives. In contrast, the free pyrimidines are not significantly incorporated, but their glycosyl derivatives are the units which are incorporated into the polynucleotides. A correlation between the general metabolic picture and the results of chemotherapeutic testing might be drawn from the fact that definite *in vivo* growth inhibitory effects have been found in the case of certain purines, but not as regards purine nucleosides. On the other hand, simple pyrimidines have been generally ineffective, while growth inhibitory activities have been found with certain pyrimidine nucleosides.<sup>22</sup> This vague and tentative correlation is based on too few examples to dignify it by calling it a conclusion. It does lend encouragement to the hope that some of these compounds are exerting their growth-inhibitory activities in a wholly approved antimetabolite manner, that is, by interfering with metabolic reactions which do, or at least can, take place in the cell.

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# THE CHEMISTRY AND BIOCHEMISTRY OF PURINE ANALOGS

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An historical account of the discovery and development of 6-mercaptapurine and its near relatives may serve the double purpose of providing both a frame of reference for subsequent studies and an introduction to the general methods of synthesis which are applicable to the preparation of purine analogs.

One of a large series of chemical relatives of the natural purine and pyrimidine bases of the nucleic acids, 6-mercaptapurine, has been investigated for a variety of biological activities.<sup>1, 2, 3</sup> This program of study was initiated for the insight it might provide into details of nucleic acid metabolism, and because it appeared probable that new chemotherapeutic agents would result. A number of lines of investigation had suggested that considerable selectivity might be expected of antimetabolites related to nucleic acid constituents. Thus, a variety of purine and pyrimidine requirements by diverse microorganisms had been discovered,<sup>4-9</sup> implying not only the natural existence of biochemical pathways for the incorporation of the preformed basic constituents of the nucleic acids, but also species differences in their occurrence. It was furthermore evident by the time these studies were initiated (1942), that there was an intimate relationship between folic acid (*Lactobacillus casei* factor) and purine and thymine metabolism,<sup>10, 11</sup> in which the former was believed to play an enzymatic role.<sup>10</sup> This finding recalled earlier studies relating purines to the metabolism of *p*-aminobenzoic acid (*p*AB) by way of their effects on sulfonamide inhibition of microorganisms,<sup>12-14</sup> although the role of *p*AB as a moiety of the folic acid molecule was not yet appreciated. It thus became apparent that a program of study of antimetabolites related to the purines and pyrimidines might profitably include provision for antifolic acid effects.

In order to implement this study, a program of chemical synthesis and a reference biological system were established simultaneously. The choice of *Lactobacillus casei* as a biological system was based on the alternative nature of its folic acid or thymine-purine requirement. This characteristic allowed an analog to be tested for its ability to simulate or antagonize folic acid, thymine, and purines in a single test through use of a few variants of the same medium.<sup>1, 2, 15</sup> This system served as a guide to the synthesis of analogs with the desired types of biochemical mechanisms of action, and as a reference system to which other biological systems could be compared. The availability of the chemical structure-biological activity pattern for this reference organism has greatly simplified the task of working out structure-activity correlation patterns for each of the succeeding biological systems which has been investigated.

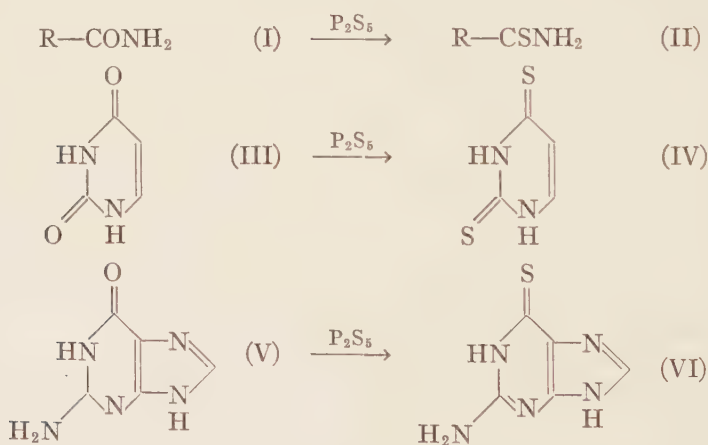
The program proposed a systematic study of the relationship between chemical structure and biological activity in order to determine the biochemical significance of each chemical feature. Each of the natural purine and pyrimidine bases has served as a starting point, and the significance of each atom has



been investigated in turn by the synthesis and study of variants of the original structure.<sup>1, 2, 15-52</sup>

It was found early in the program that a number of generalizations concerning structure-activity relationships could be formulated. An increase in the number of functional groups (such as 6-substitution in a disubstituted pyrimidine or 8-substitution in a 2,6-substituted purine) diminishes activity whether this increase be growth-promoting or inhibitory.<sup>15, 42</sup> The replacement of an atom of the ring skeleton by a suitable element often produces an antimetabolite.<sup>6, 9, 53</sup> Finally, certain changes in functional groups, such as the replacement of oxygen by nitrogen or sulfur, lead regularly to antagonists.<sup>42</sup>

The line of investigation which led eventually to 6-mercaptapurine and thioguanine began with studies of mercaptopyrimidines. Thus, thiouracil and thiothymine were found to be competitive antagonists of uracil and thymine,<sup>54</sup> and the preparation of thiocytosine<sup>20</sup> and thiopurines became of obvious interest. Some of the latter, *e.g.* 2-thioxanthine,<sup>55</sup> were known or preparable by known methods. However, methods of access to the desired 6-mercapto derivatives were not at hand and the study of various alternatives was undertaken.

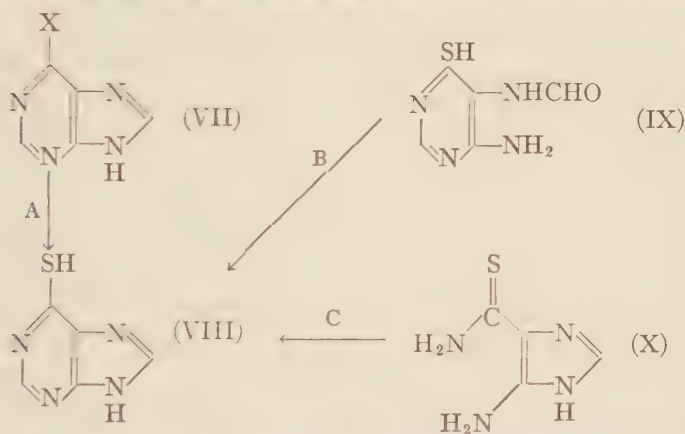


The conversion of an amide (I) to a thioamide (II) through the action of phosphorus pentasulfide was a known reaction, and the view of uracil (III) as a cyclic amide suggested trial of this reaction on uracil and structurally related condensed pyrimidine systems such as the quinazolines and purines. Physical properties, in the main solubility in suitable solvents, appear to be of prime importance to the success of this reaction. Thus, it was fruitful with many pyrimidines, and led to a considerable series of new dithio<sup>16</sup> and thio-amino derivatives.<sup>20, 22</sup> It also eventually provided thioguanine (VI) and 6-mercaptapurine,<sup>45</sup> but its application to the purines involved a number of unexpected difficulties which were overcome only after varied and persistent trial.

Thioguanine was first prepared in 1948 after several attempts. This preparation provided sufficient material for microbiological studies which confirmed

its activity as a purine antagonist.<sup>42</sup> However, repeated failures in attempts to repeat this preparation prevented biological studies of a broader scope. Attention was then turned to the preparation of 6-mercaptapurine by treatment of hypoxanthine with pentasulfide, and this procedure proved somewhat more successful. The microbiological effects were similar to those of thioguanine and consistent with the view that this substance also was an active purine antagonist. The preparation of 6-mercaptapurine provided further insight toward the solution of the technical difficulties involved in this reaction, and thioguanine also shortly became available for further study and for tumor trials.

The synthesis of 6-mercaptapurine may be used to illustrate the general methods which may be applied to the preparation of specific purines. The reaction just discussed is illustrative of a transformation reaction (A). The earliest, and still perhaps the most important route, begins with hypoxanthine



(VII, X = OH), which is reacted with phosphorus pentasulfide to effect a replacement of oxygen by sulfur.<sup>45</sup> A variant of this reaction, in which 6-chloropurine (VII, X = Cl) is reacted with sodium hydrosulfide has been used for the specific purpose of introducing S<sup>35</sup>.<sup>56</sup>

The purine ring is a fused system consisting of pyrimidine and imidazole moieties, with two carbon atoms shared in common. It is, in fact, an imidazolo (4, 5-d) pyrimidine, and it is illuminating to regard it as a hybrid with both imidazole and pyrimidine parentage. The methods for the total synthesis of purines hinge on this concept, and one may complete the fused system from either an imidazole or from a pyrimidine. The route of broadest applicability is via a pyrimidine, following the methods developed by Traube.<sup>57, 58</sup> In this scheme, a 4,5-diaminopyrimidine is synthesized and, by formylation, converted to a 5-formamido-4-aminopyrimidine, followed by cyclization of the imidazole ring. This type of reaction is illustrated by B of the chart shown above. The compound 4-amino-5-formamido-6-mercaptopyrimidine (IX) is cyclized to 6-mercaptapurine by heating of the sodium salt.<sup>56</sup> This reaction is useful for the synthesis of 8-C<sup>14</sup>-6-mercaptapurine, since it allows for the intro-



duction of the isotopic formyl group in a late step in the reaction sequence.

The alternative method for total synthesis of purines, *via* a suitable imidazole derivative, also has been explored for the synthesis of 6-mercaptopurine. Thus 4-aminoimidazole-5-thiocarboxamide, on treatment with formamide, gives 6-mercaptopurine via reaction C, a reaction which at present appears to be of academic interest only.

Thioguanine and 6-mercaptopurine were submitted for testing as possible antitumor agents in January 1951. In the first trial, 6-mercaptopurine gave a negative result (which was, however, on the borderline of scoring). Thioguanine had an unexpectedly high toxicity on repeated dosing (*cf.* Philips<sup>59</sup>), and several experiments were required before a tolerated dose was determined. Repetition of the experiments with 6-mercaptopurine established it as a tumor inhibitor of the  $\pm^{60, 61}$  class, and Law<sup>62</sup> reported its activity against L1210 leukemia. However, the observations of Clarke<sup>61</sup> on the failure to grow transplants of tumors from treated animals and the discovery of regressions of Sarcoma 180 subsequent to therapy indicated an unusual type of activity for this new antimetabolite.

The clinical studies of Burchenal *et al.*<sup>63</sup> were in accord with the preclinical work in establishing a mode of action differing from that of the antifolic acids, with consequent lack of cross-resistance. These studies suggested a useful role for the drug in the treatment of acute leukemia and led to the wider clinical studies which are reported in the present volume.

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## MICROBIOLOGICAL EFFECTS OF 6-MERCAPTOPURINE

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The theory that antimetabolites of the purine and pyrimidine bases of the nucleic acids might attack the multiplication of cells at a very fundamental level has been the basis of our program for the past 12 years. Because of the close relationship between folic acid and the biosynthesis of the nucleic acids, this work has also dealt with folic acid antagonists. By using *Lactobacillus casei* as the test system, it has been possible to study the effects of systematic structural changes of the purines, pyrimidines, and pteridines upon specific types of biological activity: namely, the ability to simulate or antagonize folic acid and the heterocyclic constituents of the nucleic acids. This study had led to a series of specific antagonists for thymine,<sup>1-4</sup> folic acid,<sup>1-3</sup> uracil,<sup>2-3</sup> and purines.<sup>2, 3, 5, 9-16</sup>

The purine antagonist, 6-mercaptopurine,<sup>17</sup> is closely analogous in structure to hypoxanthine and adenine, since all are 6-monosubstituted purines. The growth of *L. casei* is inhibited by 6-mercaptopurine,<sup>15</sup> and growth can be restored by any of the four physiological purine bases,<sup>16</sup> as shown in FIGURE 1. At concentrations of 120  $\gamma$  per ml. of 6-mercaptopurine, adenine and xanthine appear to restore growth better than guanine or hypoxanthine. Because of the relative insolubility of this inhibitor at the pH of the *L. casei* medium, it is not possible to increase its concentration to the point where one might expect to observe a pronounced specificity in the reversing agent, as in the case of high concentrations of 2,6-diaminopurine, where only adenine reverses the inhibition.<sup>3, 13</sup>

The effects of the nucleosides and nucleotides upon the inhibition of *L. casei* by 6-mercaptopurine are shown in TABLE 1. The nucleosides restore growth slightly but not as well as the corresponding free purines. On the other hand, adenylic and guanylic acids\* appear to be as effective as adenine and guanine on a molar basis. These results are in accord with the conclusions previously deduced from growth,<sup>12</sup> reversal,<sup>13</sup> and incorporation studies,<sup>18, 19</sup> that the free purines are not metabolized via their ribosides in *L. casei*, but that the "b" isomers of adenylic and guanylic acids closely resemble the free purines in their utilization.

There is a close resemblance between the behavior of thioguanine and 6-mercaptopurine in *L. casei* in that 10  $\gamma$  per ml. of any of the four physiological purines will restore growth completely in the presence of 100  $\gamma$  per ml. of thioguanine.<sup>16</sup>

A useful tool for the investigation of the mechanism of action of a drug is the study of mutants resistant to the action of the compound. Since resistance is presumably attributable to some alteration in the metabolism of the organism which permits it to survive in the presence of the drug, some clue to the locus of action of the inhibitor may often be obtained by studying the metabolic

\* Both the adenylic and guanylic acids were obtained from Schwarz Laboratories and the *a* and *b* isomers were present in unknown proportion.

TABLE 1  
EFFECTS OF NUCLEOSIDES AND NUCLEOTIDES ON INHIBITION BY 6-MERCAPTOPURINE\*

Supplement 0.05 $\mu$ M per ml.	Per cent change in titer
None	-80
Adenosine	-60
Inosine	-70
Xanthosine	-60
Guanosine	-65
Guanylic	-20
Adenylic	-20

\* In OFA medium<sup>2</sup> containing 0.6  $\mu$ M of 6-mercaptopurine per ml.

changes which accompany resistance. A strain of *L. casei* which is resistant to the action of 6-mercaptopurine (MPR strain) was isolated and found to differ from the wild strain in several respects.<sup>20, 21</sup> The resistant mutant has a requirement for folic acid which is about twice as great as that of the wild strain.<sup>21</sup> In a medium free of folic acid, the wild strain can grow on thymine and any of the four purine bases: adenine, guanine, xanthine, and hypoxanthine (FIGURE 2). The MPR mutant, on the other hand, can not use hypoxanthine at all, uses adenine poorly but still grows well on guanine or xanthine (FIGURE 2). The ribosides of adenine and hypoxanthine, which are poorer growth promoters than the corresponding purines for the wild strain, are even poorer for the mutant (FIGURE 2).

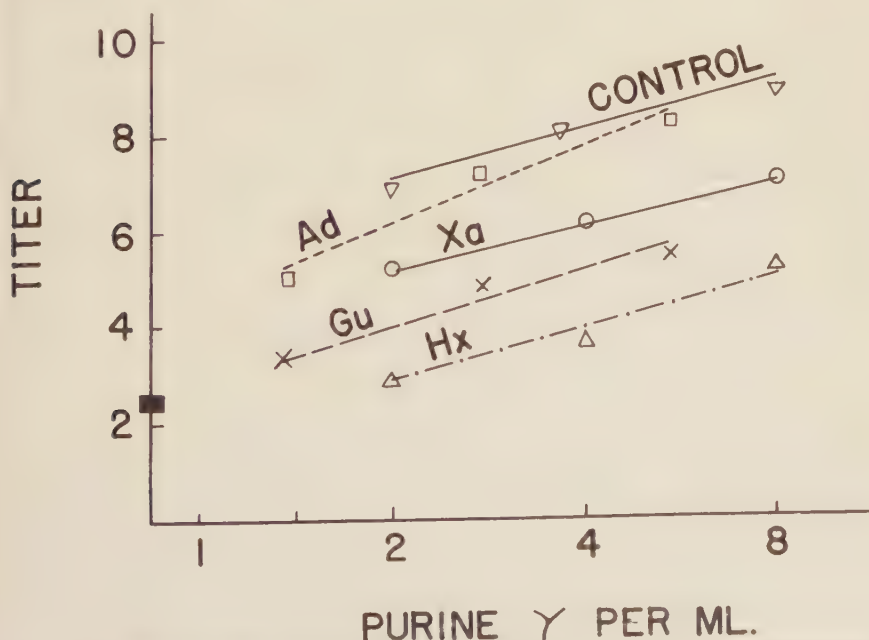


FIGURE 1. Effects of purines on the inhibition of *L. casei* by 120  $\gamma$  per ml. of 6-mercaptopurine in OFA medium. Ad, adenine; Xa, xanthine; Hx, hypoxanthine; Gu, guanine.



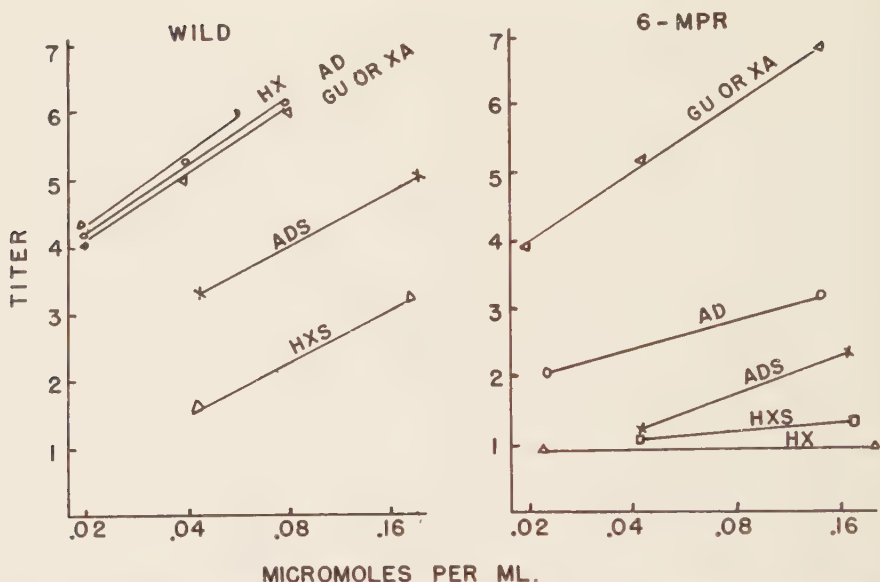
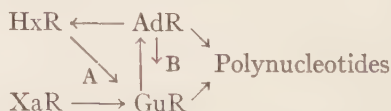


FIGURE 2. Effects of purines and ribosides on the growth of the wild and 6-mercaptapurine-resistant strains of *L. casei* in a medium containing 1  $\gamma$  per ml. of thymine. Ad, adenine; Hx, hypoxanthine; Gu, guanine; Xa, xanthine; AdS, adenosine; HxS, hypoxanthine riboside.

There is also an interesting difference between the two strains in the utilization of adenylic acid *b*. Whereas the wild strain grows well with either guanylic acid *b* or adenylic acid *b*, it cannot use adenylic acid *a* to any considerable extent<sup>19</sup> (FIGURE 3). The MPR strain does not differentiate between adenylic *a* and *b* (FIGURE 3). Neither is used well, although guanylic acid supports growth normally (FIGURE 3).

On the basis of these differences in the purine metabolism of the two strains, we were led to postulate the following scheme for the interconversion of the purine moieties in *L. casei*.<sup>21</sup> This scheme is greatly oversimplified and is meant to serve only as a working hypothesis.



Since adenine and guanine must be present in the nucleic acids, and since any of the four purines can support growth, interconversion\* can be inferred.<sup>12</sup> The scheme postulates that the purines are first converted to some more complex form, perhaps at a nucleotide level, before interconversion can occur. This level is indicated as AdR, GuR, etc. The assumption is made that there are two pathways for the conversion to AdR  $\rightarrow$  GuR, the main one being via HxR and pathway A, the minor one via some other intermediate on pathway B. If 6-mercaptapurine is interfering with pathway A, blocking the conver-

\* Interconversion of the purine moieties in *L. casei* has been confirmed by studies with labeled purines.<sup>22,24</sup>

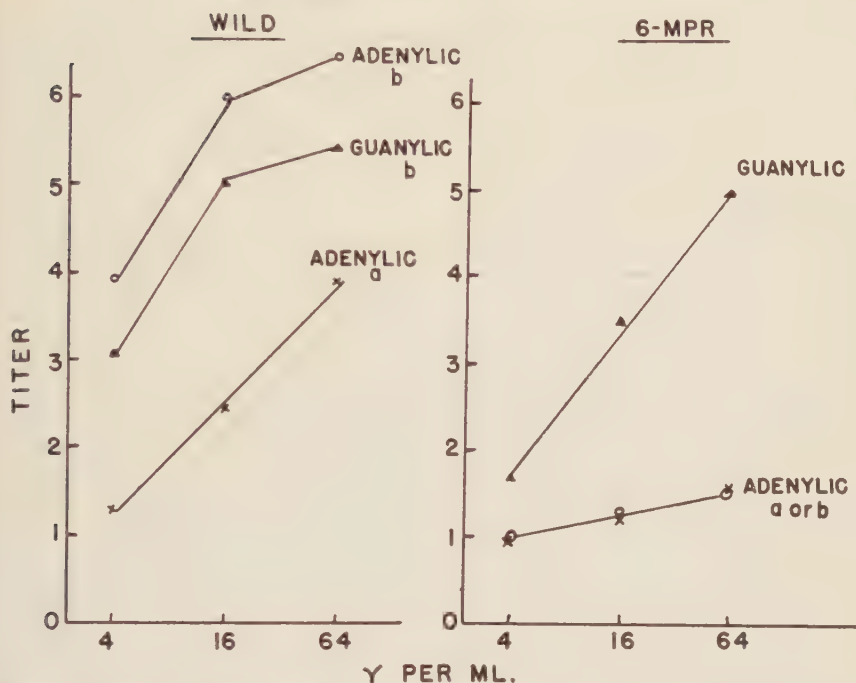


FIGURE 3. Effects of nucleotides on the growth of the wild and 6-mercaptapurine-resistant strains of *L. casei* in a medium containing 1  $\gamma$  per ml. of thymine.

sion of HxR to GuR, then the resistant organism may be assumed to be lacking this pathway and to be using the alternate pathway B for the conversion of AdR to GuR. Such a resistant mutant would be expected to be unable to utilize HxR and to make poor use of AdR. The experimental facts are in accord with this theory, since the mutant can not grow on hypoxanthine, inosine, or inosinic acid and utilizes adenine, adenosine, or adenylic acids *a* and *b* to a much smaller extent than does the wild strain. Moreover, one might expect that, when given adenine, the MPR strain might form some HxR which would then accumulate in the medium since it cannot serve as a growth source. It has been found that when this mutant is grown with thymine and 8- $C^{14}$ -adenine, the riboside of hypoxanthine, as well as free hypoxanthine, does indeed accumulate in the medium.<sup>25</sup> This is not taken to mean that inosine itself is the true intermediate HxR. Further investigation is in progress to attempt the isolation of the intermediate.

Experiments have also been carried out in collaboration with Doctors M. E. Balis and G. B. Brown\* to determine the effect of 6-MP on the interconversion of the purine moieties in the wild strain of *L. casei*. Results indicate that 6-mercaptapurine does have a marked inhibitory effect on the conversion of adenylic acid *b* to the guanine moiety of RNA, whereas the effect on the conversion of free adenine to RNA guanine is probably not great enough to be significant.

\* Unpublished experiments

TABLE 2  
EFFECTS OF INHIBITORS ON TWO STRAINS OF *L. casei*

Compound	Concn. $\gamma$ per ml.	Per cent change in titer	
		Wild	MPR mutant
6-Mercaptopurine.....	100	-85	0
6-Thioguanine.....	100	-40	0
2,6-Diaminopurine.....	10	-92	-70
8-Azaadenine.....	5	-82	-74
Purine.....	10	-91	-85
8-Azaguanine.....	0.1	-83	-70
A-Methopterin.....	0.0001	0	-56
A-Methopterin.....	0.0005	-80	-97

The MPR strain of *L. casei* is cross-resistant to thioguanine, indicating that the mechanism of action of 6-mercaptopurine and thioguanine is probably the same in this microorganism (TABLE 2). However, the mutant is still susceptible to attack by other purine antagonists.<sup>21</sup> Thus, for example, 2,6-diaminopurine, 8-azaadenine, and purine, all of which behave like adenine antagonists in *L. casei*, are almost equally inhibitory for the mutant and wild strains (TABLE 2). The same thing is true of 8-azaguanine. The MPR mutant also remains sensitive to the antifolics such as A-Methopterin. Since the mutant has a requirement for folic acid which is twice that of the wild strain, it is not surprising to find that A-Methopterin is about twice as inhibitory for the mutant (TABLE 2). If increased resistance to 6-mercaptopurine should be associated with increased sensitivity to A-Methopterin in the leukemic cell, this association might render the consecutive use of the two agents of greater value.

The fact that the development of resistance to one drug may render the organism more susceptible to another is demonstrated by experiments which involve another mutant of *L. casei*. The metabolism of the 2,6-diaminopurine-resistant strain of *L. casei* (DPR strain) has been studied in some detail.<sup>16, 26</sup> This DPR mutant is more susceptible to inhibition by 6-mercaptopurine than is the wild strain (TABLE 3). One of the metabolic differences between the DPR mutant and the wild strain is the inability of the DPR strain to utilize adenine well.<sup>26</sup> By making use of the fact that adenine can protect the wild strain from the inhibitory effects of 6-MP without protecting the MPR strain (TABLE 3), it has been possible to remove the MPR strain from a mixed culture of the two strains.

TABLE 3  
EFFECT OF ADENINE ON THE TOXICITY OF 6-MERCAPTOPYRINE

Supplement to medium $\gamma$ per ml.	Acid production ml. of 0.1 N per 10 ml.	
	Wild	DPR mutant
None.....	6.8	6.6
160 $\gamma$ 6-Mercaptopurine.....	2.0	0.5
160 $\gamma$ 6-Mercaptopurine + 2 $\gamma$ Adenine.....	4.9	0.7



TABLE 4  
SELECTIVE DESTRUCTION OF A DIAMINOPURINE-RESISTANT STRAIN OF *L. casei*

	Number of bacteria per ml.	
	After 1st transfer*	After 2nd transfer*
Total.....	$2.5 \times 10^8$	$3.1 \times 10^8$
DPR strain.....	$2.6 \times 10^7$	$<10^4$
Wild strain.....	$2.2 \times 10^8$	$3.1 \times 10^8$

\* OFA Medium contained 5  $\gamma$  per ml. of 6-Mercaptopurine and 2  $\gamma$  per ml. of adenine.

A liquid medium containing 0.05  $\mu\text{g}$ . of folic acid, 5  $\gamma$  of 6-MP and 2  $\gamma$  of adenine per ml. was seeded with an equal number of cells of the wild and DPR strains of *L. casei*. After two days of incubation, 0.1 ml. of the culture was transferred to 10 ml. of fresh medium and incubated another two days. The population was determined after each incubation period by plating on two types of agar, one of which would permit both strains to grow, the other containing a high concentration of 2,6-diaminopurine, which would permit only the DPR mutant to grow. The concentration of the wild strain could then be determined by difference. It will be seen from TABLE 4 that, two days after the first transfer, 84 per cent of the population was wild strain and only 16 per cent was the DPR mutant. After the second transfer almost the entire population consisted of wild strain,  $3.1 \times 10^8$  organisms per ml., while few if any DPR mutant cells remained. The results of the experiment suggest that one can selectively protect the normal cell against a drug which is inhibitory to both the normal and mutant cell, and that a cell which has become resistant to one agent may show increased vulnerability to another because of the very alterations in its metabolic pathways which have rendered it resistant to the first drug.

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# ACTIVITIES OF 6-MERCAPTOPURINE AND RELATED COMPOUNDS ON EMBRYONIC AND REGENERATING TISSUES OF *RANA PIPIENS*\*

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The developing embryo might be expected to be sensitive to antimetabolites which affect nucleic acid biosynthesis because of the rapid cellular division and progressive differentiation which development involves. Studies of the effects of the antagonists on this system might be expected not only to cast light on the biochemistry of embryonic development, but also to contribute information concerning the mechanisms of action of the analogues involved and, in these ways, to contribute to the broad program of synthesis and study of analogues of nucleic acid derivatives in these laboratories.<sup>1-3</sup>

The *Rana pipiens* embryo was chosen as a test object because it presents a minimum of technical difficulties and allows a rapid screening with well-marked criteria of activity. In addition, and perhaps more important for the present purposes, is the availability for study of the early stages of morphogenesis. Preliminary studies<sup>4, 5</sup> indicated that this embryo is indeed sensitive to antagonists of the nucleic acid series, and called attention to 6-mercaptopurine as outstanding in its ability to inhibit the earlier stages of differentiation.

The developing embryo contains many differentiating organs and biochemical systems. The resultant interrelationships are consequently of great complexity. In order to minimize some of these effects, and to provide a system in which a separation of differentiation from cell multiplication can be achieved to some degree, advantage was taken of the amphibian's capacity for regeneration of a lost member. When the tail of a tadpole is severed, the tissue response involves the following steps: (1) wound closure; (2) demolition of damaged cells; (3) dedifferentiation and or mobilization of cells to provide for new tissues; (4) formation of the blastema or regeneration bud; (5) growth of the blastema; and (6) redifferentiation of the young regenerate. The first four steps, involving morphological and biochemical reorientation, occur over a period of several days and with a minimum of concomitant cell division. Preliminary studies<sup>6</sup> revealed that this phase of regeneration was susceptible to the action of various antimetabolites and emphasized the activity of 6-mercaptopurine on relatively undifferentiated cells.

## *Materials and Methods*

Eggs were obtained from female *Rana pipiens* by induced ovulation.<sup>7</sup> The compounds were tested, as previously described,<sup>4, 5</sup> over a concentration range of 1 to 50 mgm. per cent. Developmental progress was noted in terms of Shumway stages<sup>8</sup> and by comparison with the control groups of animals. Reversal studies were carried out by adding to the culture media various concentrations of metabolites in the presence of the inhibitor. All animals were kept

\* This study was supported by a grant from the Charles F. Kettering Foundation to the Wellcome Research Laboratories.



in an incubating refrigerator at 18° C. 6-Mercaptopurine was also tested on ova which matured in females maintained under starvation conditions.

Sample embryos were prepared for histological examination according to the method of Drury,<sup>9</sup> sectioned at 10 micra, and stained with Galigher's hemotoxylin<sup>10</sup> and eosin.

Tails were amputated from tadpoles (2.0 to 2.5 cm. in length) in the manner described elsewhere.<sup>6</sup> The animals were immersed for five hours in 0.5 per cent sulfadiazine (to prevent infection during wound healing) before being placed in test solutions which ranged in concentration from 0.1 to 100 mgm. per cent. Solutions were changed every third day. The animals were kept at 18° C. and were fed boiled spinach *ad libitum*. On the seventh day following amputation, measurements of blastema length were made by means of an ocular micrometer and data were recorded in terms of ocular micrometer units.

Reversal studies were carried out, as in the embryo screen, by the administration of various metabolites in the presence of the analogue.

### Results

Thioguanine, 8-azaguanine, and 2,6-diaminopurine did not affect development of the embryo at any of the concentrations tested. Developmental rates and gross morphology of the test embryos were comparable with those of the control groups.

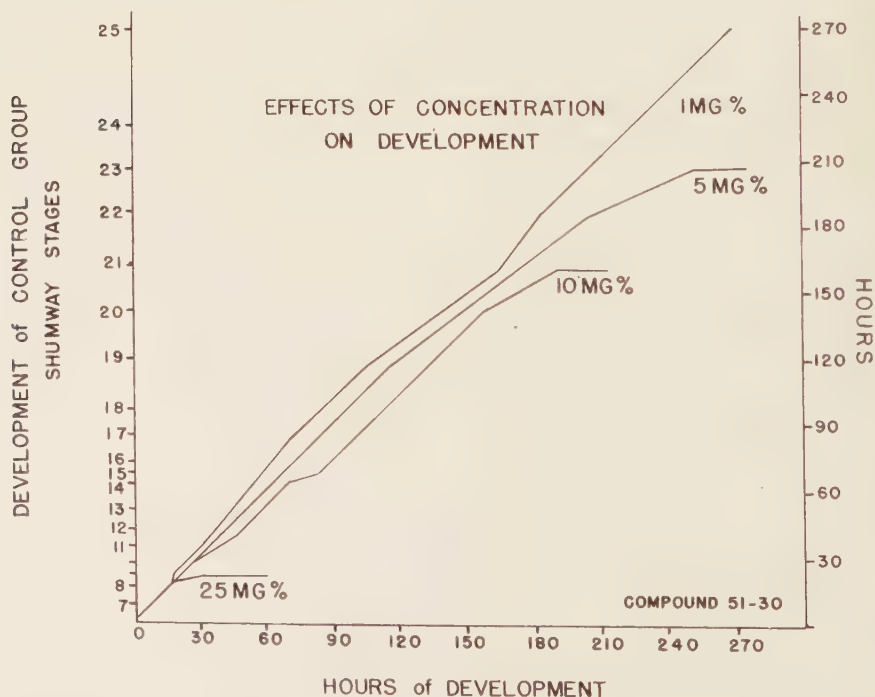


FIGURE 1. Effects of various concentrations of 6-mercaptopurine on *Rana pipiens* embryos immersed at the two cell stage.

A slight retardation of development was effected by 6-mercaptopurine at Shumway stages 11 to 14 (gastrula and neurula) and moderate retardation after hatching (Shumway stages 20 to 23) in eggs obtained from well-nourished females. Grossly evident were interference with gill and opercular formation and poor myotomic differentiation. These effects were produced only at the highest concentration (50 mgm. per cent). A delay in treatment until Shumway stage 8 or later resulted in the loss of inhibitory activity. Of the eight naturally occurring purines, nucleosides, and nucleotides tested, only adenine and adenylic acid gave a partial reversal of the inhibition.

Ova which matured in females maintained under starvation conditions exhibited an increased susceptibility to inhibition by 6-mercaptopurine. A ten-fold decrease in the effective concentration was noted along with retardation of development at all four of the critical periods,<sup>5</sup> *i.e.* blastula, gastrula, neurula, and the broad period from hatching to operculum formation (FIGURE 1). Inhibited embryos had the gross and microscopic appearance usually associated with interference with the normal developmental pattern, *e.g.* microcephalia, poor eye development, aberrant gill formation, *etc.*<sup>5</sup>

Thioguanine and 6-mercaptopurine were found to have notable activity on

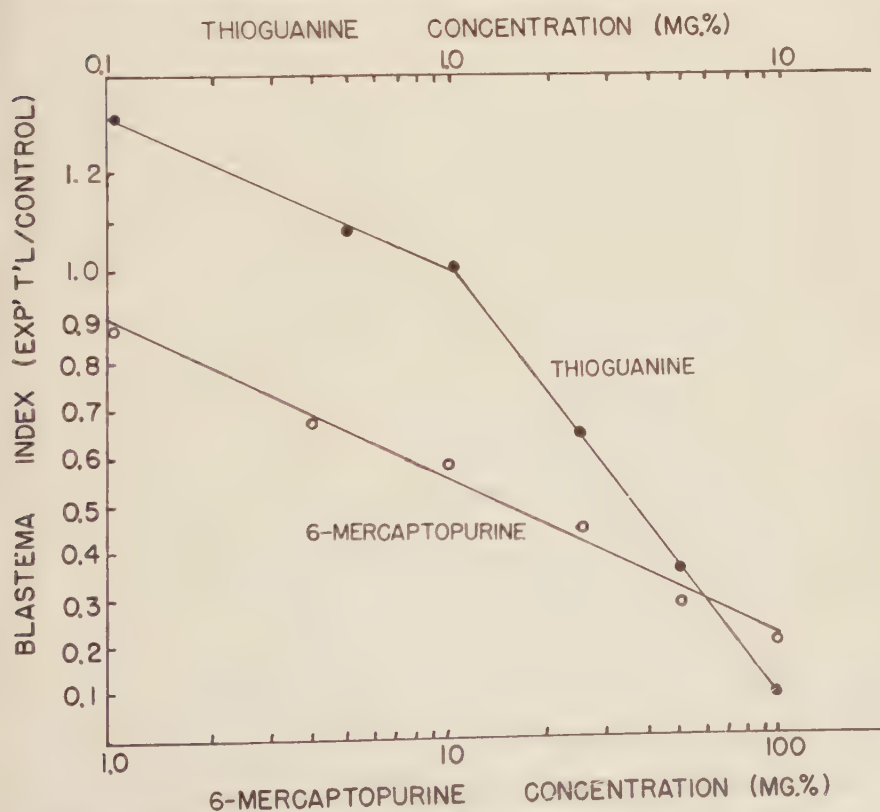


FIGURE 2. Effects of 6-mercaptopurine and thioguanine on blastema formation in *Rana pipiens* tadpoles.

TABLE 1

Compound	M/A*	Blastema** index	% Reversal
6-Mercaptopurine ( $5.9 \times 10^{-4}$ M.).....	—	0.56	—
+ Adenine.....	3.2	0.76	45.5
	4.8	0.72	36.4
+ Adenylic Acid.....	2.4	0.75	43.2
	3.6	0.86	68.0
+ Guanylic Acid.....	3.5	0.76	45.5

\* M/A—Molar ratio of metabolite to antimetabolite.

\*\* Blastema index—length experimental blastema/length control blastema.

TABLE 2

Compound	M/A	Blastema index	% Reversal
Thioguanine ( $2.8 \times 10^{-4}$ M.).....	—	0.25	—
+ Adenine.....	6.8	0.68	57.2
+ Adenylic Acid.....	5.1	0.51	34.7
+ Guanylic Acid.....	5.0	0.53	37.2
+ Inosine.....	6.7	0.37	16.0

the regenerating tail blastema. A two-phase dose response curve was obtained by plotting the blastema index (length of experimental blastema/length of control blastema) against the log of the concentration of the analogue (FIGURE 2). The first response varied from stimulation of blastema formation (thioguanine curve) to slight inhibition. This effect was followed by a sharp change in the slope of the curves together with marked inhibition of blastema formation. The inhibition with thioguanine occurred over a narrow concentration range (1 to 10 mgm. per cent) with maximum inhibition produced at one tenth the dose required for 6-mercaptopurine. At this 7-day measuring period, 8-azaguanine and 2,6-diaminopurine were inactive, but both compounds showed a slight activity two weeks after treatment was started.

The effects of 6-mercaptopurine were reversed incompletely by adenine and, apparently, competitively by adenylic acid (TABLE 1); guanylic acid was as effective in reversing inhibition as adenine. The results with thioguanine were somewhat different. Adenine appeared to be more effective than either adenylic or guanylic acid in sustaining blastema formation (TABLE 2), and inosine had a slight activity in reversing inhibition. It is interesting to note that, in the case of thioguanine, higher molar concentrations of the metabolites were required to produce reversal than in the case of 6-mercaptopurine.

### Discussion

The activity of 6-mercaptopurine in both embryological systems provides insight into its mode of action. The first detectable biochemical changes in the embryo occur at late blastula (Shumway stage 9) when the embryo has achieved its most primitive form of differentiation. Fewer than 5 per cent of the analogues tested were active at this early stage. When treatment with 6-mercaptopurine was begun at the two-cell stage of development, a marked



inhibition at stage 8 was observed. However, when treatment was postponed to stage 8 or later, no effects on development were seen. Its activity accordingly appears to be associated in some way with the very early and primitive biochemical activities of the embryo. The increased activity of 6-mercaptopurine in eggs from starved females and the inability of thioguanine to act on normal ova may possibly be explained by the depletion of cytoplasmic stores, including the normally large complement of purines, recently noted in starved ova.<sup>11</sup> This explanation is consistent with the observed ability of adenine and adenylic acid to block the effects of 6-mercaptopurine.

The gross and histological abnormalities noted in the 6-mercaptopurine treated groups were nonspecific in nature and similar to the abnormalities produced by a variety of chemical and physical means. They may reflect simply an interference in the normal developmental pattern and time sequence. The retardation of development just preceding gastrulation has long been known to produce the same variety of lesions noted in these experiments.<sup>12</sup>

Blastema formation involves the dedifferentiation (morphological and biochemical) of existing cells preceding cell multiplication. It is interesting to note, at this point, that 8-azaguanine and 2,6-diaminopurine evidence some activity during the stage of cellular proliferation, but little inhibition of blastema formation.

In both systems, it is evident that the activities of 6-mercaptopurine and thioguanine are directed primarily against relatively undifferentiated cells. These findings are consistent with the observations of Thiersch,<sup>13</sup> which suggest an all-or-none effect on the rat embryo at the time of implantation, and failure of 6-mercaptopurine to affect later development. Similarly, only rapidly dividing and relatively undifferentiated neoplastic tissues have evidenced a selective sensitivity to this agent. It is not possible, at the present time, to evaluate the relative importance of the rate of cell division and the state of differentiation involved. However, the effectiveness of the two mercaptopurines in the inhibition of blastema formation, where cell division is minimal, does suggest that the state of differentiation of the cell may affect its sensitivity to these substances.

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# BIOLOGICAL ACTIVITIES OF 6-MERCAPTOPURINE: EFFECTS ON *STREPTOCOCCUS FAECALIS*\*

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The inhibitory effects of 6-mercaptopurine (6-MP) on two strains of *Lactobacillus casei*, and the rapid development of resistance to 6-MP by *L. casei* have been demonstrated by Elion *et al.*<sup>1, 2</sup> In an attempt to delineate further the modes of action of this antimetabolite, a study has been made to determine the effects of 6-MP on *Streptococcus faecalis* ATCC 8043, *S. faecalis*/A (resistant to 4-amino-10-methyl-PGA) and *S. faecalis*/MP (resistant to 6-MP).

Previous studies revealed a number of quantitative differences between the wild strain of *S. faecalis* and *S. faecalis*/A.<sup>3</sup> For example, *S. faecalis*/A exhibited a threefold decrease in PGA requirement, and a 500-fold decrease in pteric acid requirement in a medium containing purines. Furthermore, the mutant appeared to be utilizing, in the absence of pteroylglutamic acid (PGA), certain folic acid antagonists for growth.<sup>4</sup> This effect was found to be a response of the resistant organism to the PGA and/or pteric acid present in these preparations as impurities.<sup>5, 6</sup> An even more striking difference was noted between *S. faecalis* 8043 and *S. faecalis*/A in a study of the enzymatic formation of citrovorum factor (CF) from PGA. The resistant strain was approximately 20 times more efficient than the wild strain in this enzymatic process.<sup>5, 6, 7</sup> Nichol<sup>8</sup> has shown that cell-free extracts of *S. faecalis*/A were also active in forming CF from PGA, and this system was more susceptible to antifolics than was the system with the whole cells. A decrease in the capacity to carry out *de novo* synthesis of purines as reflected by growth of *S. faecalis*/A in purine-deficient media was also observed.<sup>9</sup>

The isolation of, and studies with, a strain designated as *S. faecalis*/MP will be described in this report.

*Experimental methods.* The usual microbiological assay techniques were employed in these experiments. All studies were carried out in the medium of Flynn *et al.*<sup>10</sup> designed especially for the growth of *S. faecalis*, from which adenine, guanine, xanthine, and uracil were omitted. This modified medium has been designated as F-PP medium. Supplements will be indicated with each experiment. Incubation was at 35° C. for 16 to 18 hours unless otherwise stated, and growth was measured in terms of optical density on a Coleman Junior spectrophotometer.

*S. faecalis* 8043 was maintained on Difco Folic Acid Assay medium, to which 1 mγ/ml. of PGA was added. *S. faecalis*/A was carried on Difco FAA medium supplemented with 100 γ/ml. of 4-amino-10-methyl-PGA. *S. faecalis*/MP was cultured on F-PP medium which contained 1 mγ/ml. of PGA and 500 γ/ml. of 6-MP.

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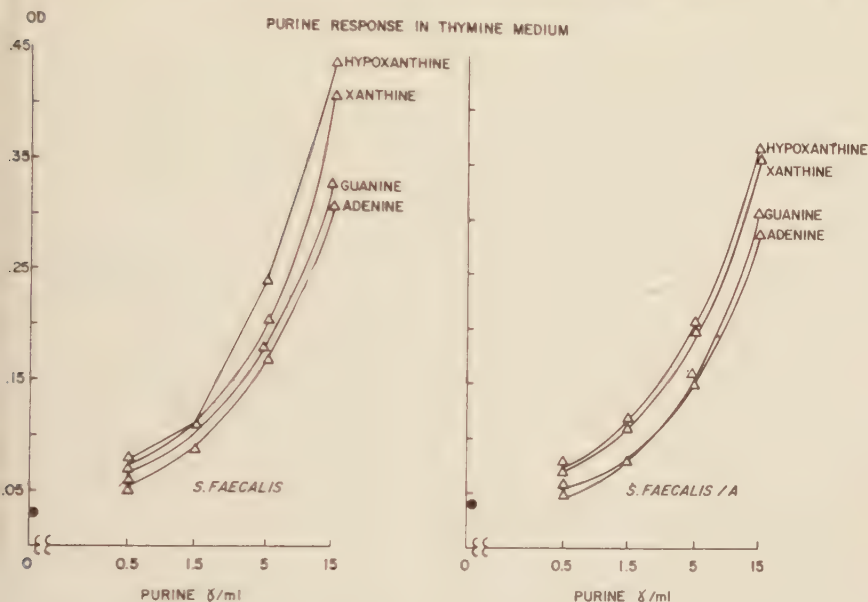


FIGURE 1. Growth response of *S. faecalis* and *S. faecalis/A* in F-PP medium plus 2.5  $\gamma$ /ml. thymine.

The isolation of *S. faecalis* MP was achieved by serial daily transfers of *S. faecalis* 8043 through increasing concentrations (ranging from 10–500  $\gamma$ /ml.) of 6-MP in the F-PP medium supplemented with PGA at 1  $m\gamma$ /ml. After 8 transfers, the organism gave maximum growth, in 16 to 18 hours, in the presence of 500  $\gamma$ /ml. of 6-MP. In other experiments, it was possible to obtain small amounts of growth of *S. faecalis* 8043 on 500  $\gamma$ /ml. of 6-MP if the incubation time were extended to 72 to 96 hours.

**Results.** The effects of adenine sulfate, guanine hydrochloride, hypoxanthine, and xanthine in F-PP medium containing either thymine (2.5  $\gamma$ /ml.) or PGA (0.2  $m\gamma$ /ml.) were investigated. The results on the thymine medium (FIGURE 1)\* agree with the report of Stokes<sup>11</sup> that the PGA requirement of *S. faecalis* could be met by a combination of thymine and one of the natural purines. *S. faecalis/A* responded in a similar manner (FIGURE 1). The ribosides of adenine, guanine, hypoxanthine, and xanthine were also effective growth substances for both organisms in this medium.

By contrast, *S. faecalis*/MP did not grow on adenine, guanine, or hypoxanthine, but exhibited an excellent response to xanthine (FIGURE 2). There was no stimulation of growth after 48 hours by adenine, guanine, or hypoxanthine. Furthermore, a mixture of any two of the three inactive purines or a mixture of all of them could not replace xanthine. Xanthosine was as effective as the free base in supporting growth of *S. faecalis*/MP.

In the F-PP medium containing PGA, the response of *S. faecalis*/MP was similar to that observed in the thymine medium (FIGURE 2). *S. faecalis* was

\* In all figures the growth obtained in the absence of a natural purine is indicated by a black, closed circle near the ordinate.



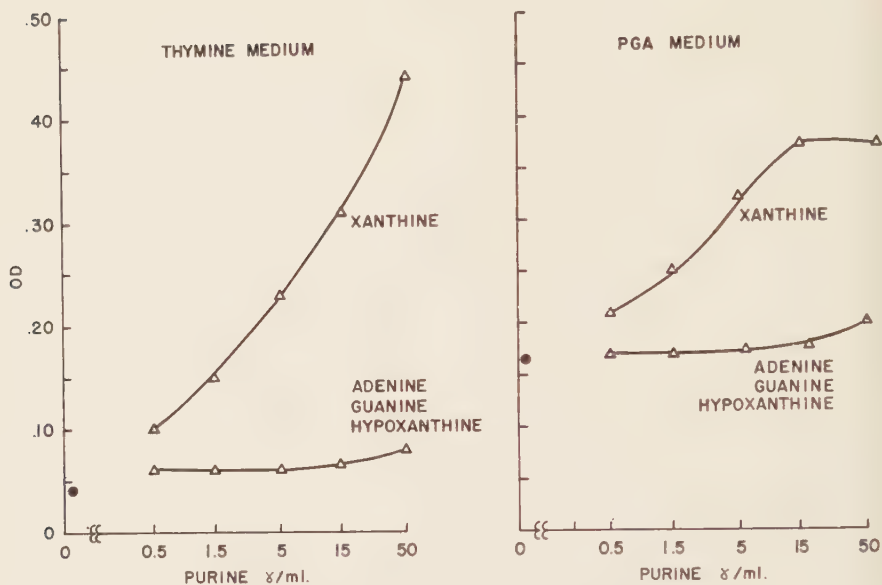


FIGURE 2. Growth response of *S. faecalis*/MP to purines in F-PP medium plus 2.5  $\gamma$ /ml. thymine and 0.2 m $\gamma$ /ml. PGA.

somewhat stimulated by low concentrations of the purines, 1.5–10  $\gamma$ /ml. (FIGURE 3), but grew very well in the absence of purines. High concentrations (50  $\gamma$ /ml.) of adenine were inhibitory. *S. faecalis*/A (FIGURE 3), on the other hand, did not grow in 18 hours in the absence of purines, and the response to the individual purines was similar to that noted in the thymine medium.

*S. faecalis* and *S. faecalis*/MP had approximately the same PGA response in F-PP medium and in the complete Flynn medium. The amount of PGA required for half-maximum growth was 0.2 to 0.3 m $\gamma$ /ml., with *S. faecalis*/MP always exhibiting somewhat more growth. *S. faecalis*/A required very little PGA in complete medium, 0.1 m $\gamma$ /ml.,<sup>4</sup> while in the F-PP medium as much as 1.0 m $\gamma$ /ml. was required to give half-maximum growth, which usually was not attained until after 48 hours of incubation. *S. faecalis*/MP also showed a 20- to 40-fold increase in resistance to 4-amino-10-methyl-PGA and 2,4-diamino-5(3'4'-dichlorophenyl)-6-methyl pyrimidine.

The alterations in the *de novo* purine synthesis and the pathways concerned with the utilization of exogenous purines of *S. faecalis*/A and *S. faecalis*/MP, as shown in the above results, suggested further investigations with purine analogs or antimetabolites.

Since *S. faecalis*/A grew poorly in F-PP medium containing low concentrations of PGA, it was not surprising that 6-MP was more inhibitory than to *S. faecalis* and *S. faecalis*/MP (TABLE 1). The greater response of *S. faecalis*/A to the natural purine bases suggested that in the presence of a stimulatory

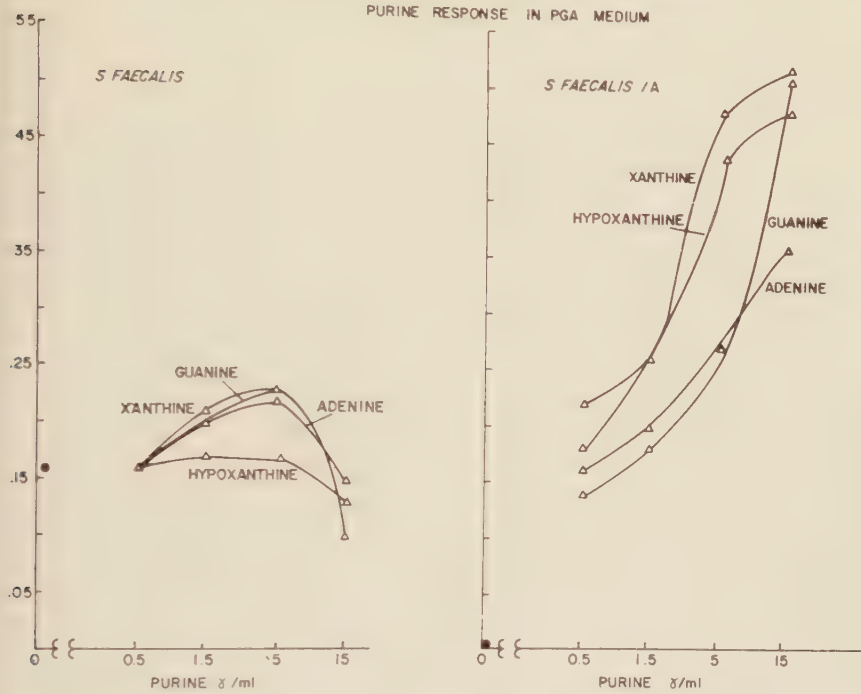


FIGURE 3. Growth response of *S. faecalis* and *S. faecalis/A* to purines in F-PP medium plus 0.2 mg/ml. PGA.

TABLE 1  
50 PER CENT INHIBITION BY 6-MERCAPTOPURINE\*

	Supplements				
	None	Adenine	Guanine	Hypoxanthine	Xanthine
	Thymine Medium				
<i>S. faecalis</i> .....	0	100	50	100	100
<i>S. faecalis/A</i> .....	0	75	75	75	75
<i>S. faecalis/MP</i> .....	0	0	0	0	>500
	PGA Medium				
	7.5	25	25	25	200
	0.5	500	200	300	1.0
<i>S. faecalis</i> .....	>500	>500	>500	>500	>500
<i>S. faecalis/A</i> .....					
<i>S. faecalis/MP</i> .....					

\* Amount of 6-MP in micrograms that produced half maximum growth in the presence or absence of 20 micrograms of supplement.

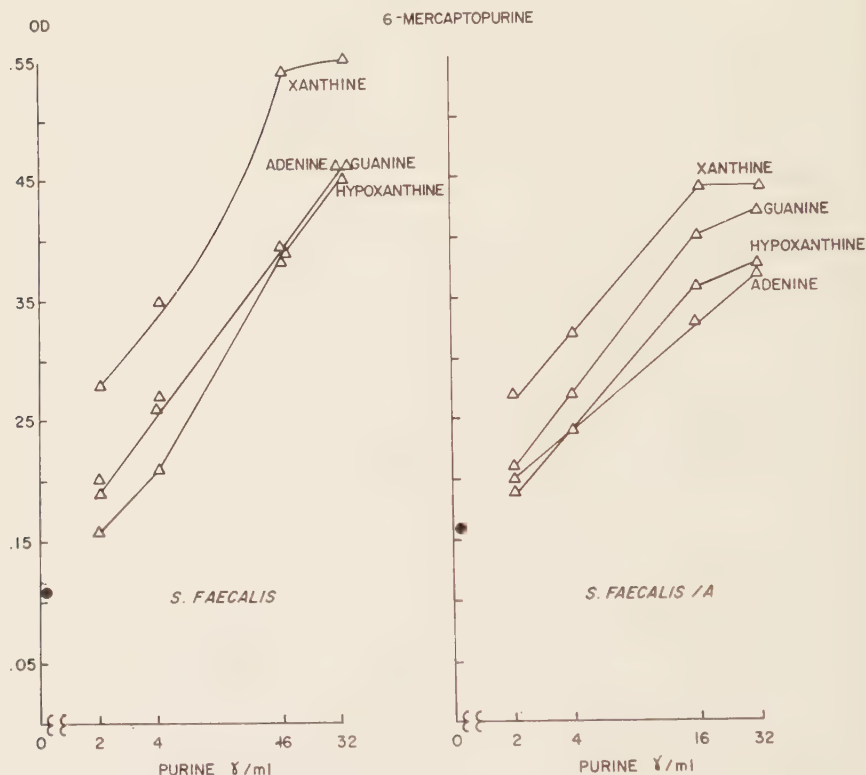


FIGURE 4. Effect of purines on 6-mercaptopurine toxicity in F-PP medium plus 2.5 γ/ml. thymine.

purine this inhibition might not be so marked, except at the specific locus of antagonism.

The inhibition of *S. faecalis* and *S. faecalis/A* by 6-MP in the thymine medium was reversed by any one of the four purine bases. In the presence of xanthine, 6-MP had no effect on the growth of *S. faecalis/MP* (TABLE 1). The effect of various concentrations of purine bases on the reversal of inhibition by 6-MP (150 γ/ml.) is presented in FIGURE 4 and, as in the above study, all purines were effective in reversing 6-MP inhibition for *S. faecalis* and *S. faecalis/A*.

When the organisms were concerned with both *de novo* purine synthesis and utilization of preformed purines, as was the case in the PGA medium, the results were somewhat different (TABLE 1). Xanthine was the most effective in reversing 6-MP toxicity for *S. faecalis*, while in the case of *S. faecalis/A*, xanthine was the only ineffective purine for the reversal of toxicity. 6-MP was not inhibitory for *S. faecalis/MP* in the PGA medium in the presence or absence of any of the purines.

The effects of various concentrations of purine bases on the reversal of 6-MP inhibition of *S. faecalis* and *S. faecalis/A* in a PGA medium are presented in FIGURE 5. In 18 hours, in the presence of 10 γ/ml. of 6-MP, increasing concen-



## 6-MERCAPTOPURINE

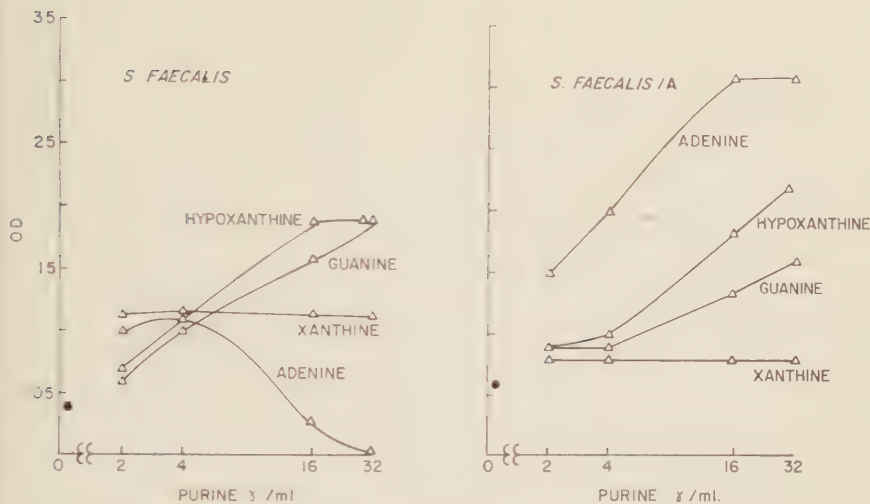


FIGURE 5. Effect of purines on 6-mercaptopurine toxicity in F-PP medium plus 0.2 mγ/ml. PGA.

trations of the purines reversed the toxicity for *S. faecalis* in the order of hypoxanthine, guanine, xanthine, and adenine and, in the case of *S. faecalis/A*, adenine was the most effective purine and xanthine was ineffective. Hypoxanthine and guanine were intermediate. However, when the tubes of this experiment were incubated for six more hours, xanthine became an effective reversing agent for *S. faecalis A*, but adenine did not overcome the inhibitory effect of 6-MP for *S. faecalis*.

Thioguanine, an analog of 6-MP, was found to be similar to 6-MP, with the notable exception that xanthine did not reverse the inhibitory effect of thioguanine for *S. faecalis* (TABLE 2). *S. faecalis/MP* was highly resistant to thioguanine in both the PGA and thymine media.

Purine (TABLE 3) was inhibitory to *S. faecalis/MP*. In the thymine medium, xanthine was slightly more effective in reversing the toxicity of purine for *S. faecalis* than it was for reversing the toxicity for *S. faecalis/MP*. In the PGA medium, the purine inhibition of both organisms was reversed by xanthine and adenine. Even though adenine was not stimulatory for *S. faecalis/MP*, it was effective in reversing purine inhibition. A similar phenomenon has been reported in 6-MP-resistant *L. casei*.<sup>2</sup>

On the *de novo* synthesis of both *S. faecalis* and *S. faecalis/MP*, 8-azaxanthine was an extremely effective inhibitor; 2 γ/ml. of azaxanthine produced 50 per cent inhibition of both cultures. Xanthine was the only reversing agent for *S. faecalis/MP*, while all the purines effectively reversed the inhibitory effect on *S. faecalis*.

In the PGA medium, 2,6-diaminopurine was inhibitory to *S. faecalis/MP* at a level of 200 γ/ml., which represents a 100-fold increase in resistance by com-

TABLE 2  
 50 PER CENT INHIBITION BY THIOGUANINE\*

	Supplements				
	None	Adenine	Guanine	Hypoxan- thine	Xanthine
	Thymine Medium				
<i>S. faecalis</i> .....	0	150	100	100	<5
<i>S. faecalis</i> /MP .....	0	0	0	0	>500
	PGA Medium				
<i>S. faecalis</i> .....	<5	50	30	15	<5
<i>S. faecalis</i> /MP .....	400	400	400	400	400

\* See footnote for TABLE 1.

 TABLE 3  
 50 PER CENT INHIBITION BY PURINE\*

	Supplements				
	None	Adenine	Guanine	Hypoxan- thine	Xanthine
	Thymine Medium				
<i>S. faecalis</i> .....	0	300	100	400	100
<i>S. faecalis</i> /MP .....	0	0	0	0	40
	PGA Medium				
<i>S. faecalis</i> .....	20	200	50	200	70
<i>S. faecalis</i> /MP .....	20	250	20	20	70

\* See footnote for TABLE 1.

parison with *S. faecalis*. Resistance was observed in *S. faecalis*/MP in the above medium to 8-azaadenine, 8-azaguanine, and 8-azahypoxanthine in the order of 5-, 100-, and 50-fold increases respectively.

**Discussion and Summary.** These studies on three strains of *S. faecalis* indicate a number of variations in regard to the biological effects of 6-MP, all of which appear to be dependent upon metabolic alterations within the mutant cultures.

In a medium where the *de novo* pathway of purine biosynthesis can be followed, as well as the incorporation or utilization of exogenous purines, it appears that *S. faecalis* prefers the *de novo* route and *S. faecalis*/A prefers the utilization of exogenous purines, while *S. faecalis*/MP is somewhat intermediate. In the case of *S. faecalis*/MP, if xanthine was present both routes were followed. If xanthine was absent, then only the *de novo* pathway was followed, since the other purines, alone or in combination, are ineffective as growth stimulants.

In the early growth phase of *S. faecalis* A in the presence of PGA and purines, 6-MP interferes with the utilization of xanthine, while, after 24 hours, xanthine becomes almost as effective as adenine, guanine, or hypoxanthine in reversing the inhibitory effect of 6-MP. Adenine does not effectively reverse 6-MP toxicity for *S. faecalis*, even up to 24 hours. In *S. faecalis*, it is possible that the affinity is greater for 6-MP, in the competition between adenine and 6-MP for an enzyme (or enzymes) or it is also possible that this effect may be a result of a combined inhibitory action of 6-MP and adenine, since high concentrations of adenine alone were inhibitory. Adenine was the most effective reversing agent of 6-MP for *S. faecalis* A, which shows that a specific alteration has occurred and, possibly with this mutant, the enzyme affinity has shifted to the metabolite and away from the antimetabolite. Also, high concentrations of adenine are not inhibitory to the organism.

The fact that xanthine played an important role in the PGA medium, both with *S. faecalis* and *S. faecalis* A when 6-MP was present and, in *S. faecalis*/MP in the absence of 6-MP, suggests that, in these microorganisms, the metabolism of xanthine or a closely related compound may be interfered with by 6-MP. The unequivocal proof of these relationships is dependent upon tracer studies.

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# THE EFFECT OF 6-MERCAPTOPURINE ON THE RAT FETUS AND ON REPRODUCTION OF THE RAT\*

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## Introduction

The synthesis of antimetabolites has permitted, in the past five years, easier and less time-consuming studies of nutritional deficiencies. These studies have rapidly increased our knowledge of fetal requirements as regards vitamins, so that mother animals can now be maintained on an almost normal diet and frequently are not seriously affected by antimetabolites. Classical examples of such experiments are the studies on riboflavin with the antimetabolite galactoflavin<sup>1</sup> and the studies on folic acid and its antagonists x-methyl and 4-amino folic acid.<sup>2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12</sup> In the course of these studies, it has become apparent that the time of antimetabolite administration is of importance, and that the fetus has definite periods of sensitivity. It is also apparent that nonlethal doses can induce malformation involving, in a typical sequence, the fetal structures, beginning with the head and descending to lower structures. The molecular structure,<sup>13, 14</sup> as well as early experiments with microorganisms, had suggested that 6-mercaptopurine (6-MP) was an antimetabolite of adenine and hypoxanthine,<sup>15</sup> but in leukemias it behaved like an antipurine.<sup>16</sup> Its action on the embryos of *Rana pipiens* was of great interest,<sup>17</sup> because 6-MP affected the frog embryo at an earlier stage of development than folic acid antagonists. In order to determine its effect on the mammalian fetus the experiments reported here were conducted.

The present experiments were conducted with the Long-Evans strain of rats, bred and reared in our own colony. The animals were maintained on the "white" breeding diet† of Long and Evans *ad libitum*. For controls and experimental work, only rats that had had at least one previous litter were used in order to avoid the irregularities of the first gestation. The animals weighed between 250 and 300 gm. and were seven to eight months old. The gestation period of this strain is 22 days. Considering the morning of massive sperm findings in the vagina as day zero, implantation of fertilized ova takes place late on the seventh day. The average number of implantations for this strain of rats is 8.6.<sup>18</sup> The average weight gain of nonpregnant rats for a period of 21 days was 6 per cent; of pregnant rats receiving tap water or placebos, 36 per cent.

## Procedure

The onset of pregnancy was determined in all animals by examining the vaginal contents for sperm. Fertilized animals were kept in single cages and periodically weighed and treated. The experimental and control rats received

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† White diet (percentages): ground whole wheat, 67.5; casein, tech., 15.0; skim milk powder, 7.5; NaCl crystallized, 0.75; Ca<sub>2</sub>CO<sub>3</sub>, 1.5; melted fat, 6.75; fish oil, (vitamins A-D, conc.), 1.0; K.I. Sol., 450 mgm. L., lettuce supplement twice weekly.

the drug or a placebo in a suspension of 10 cc. of water per kg. body weight administered with the stomach tube. Struggling of the rats and spilling were carefully avoided, and only a single rat dose was drawn up in the syringe at a time, to prevent overdosing. The *controls* consisted of one group of rats treated with tap water from the fifth to fourteenth day, and another group treated from the seventh to sixteenth day of gestation with an ineffective compound. The control rats were in all respects handled and treated as were the experimental rats, but were kept in a separate room. All rats were sacrificed under ether anesthesia on the 21st day of gestation, the day prior to expected littering. Maternal hemoglobin and sternal bone marrow were examined, the uteri removed intact, and implantations counted. The uteri were then opened longitudinally, the fetuses counted, measured and weighed, inspected for malformations, and the head cross-sectioned to detect internal hydrocephalus. At least one apparently normal and all stunted fetuses were sectioned and X-rayed, the fetal hemoglobin was determined, and implantation sites without fetuses were sectioned. Originally, each experimental and control group was started with 30 rats. In such a group, approximately 70 per cent were found to have been or to be pregnant at the time of sacrifice. Doses of 30 and especially 60 mgm./kg. were found to be toxic, resulting in the death of many animals, thus reducing the number of rats in the group, so that a second group had to be employed. Complications occurred as a sequence to fetal death, and necrosis resulted in eight cases. The complications consisted of a purulent endometritis with occlusion of the tubes and pseudocyst formation of the ovary. After each group of animals was sacrificed and sectioned, all data were summarized, averaged, and the standard deviation determined.

### *Experiments*

Groups of female rats were treated: (1) prior to mating; (2) on the fourth and fifth days of pregnancy prior to implantation; (3) On the seventh and eighth days during and immediately following implantation; and (4) On the 12th and 13th days—five and six days after implantation—to observe the effect of the drug.

### *Results*

*Controls.* As shown in TABLE 1, the control group of rats produced fetuses more than 4 cm. long with a standard deviation reaching down to 3.6 cm. In accordance with this finding, fetuses of the experimental groups measuring less than 3.5 cm. in length were recorded as "stunted."

Resorbed fetuses were observed in both control groups to the maximum of 1.7 per cent and a standard deviation of  $\pm 2.4$  per cent. In the experimental groups the number of resorptions were regarded as statistically significant only if the percentage rose above this figure without overlap of the standard deviations. The number of implantations in control and experimental groups did not differ significantly in groups treated with less than 30 mgm./kg. X-ray films of the skeleton of control and experimental rats did not reveal gross abnormalities, nor did sectioning of the fetuses at five different levels show malformations. No case of internal hydrocephalus was noted upon cutting into

TABLE 1.  
6-MERCAPTOPURINE.

	Mother rats percentage weight gain <sup>1</sup> 1/21	Fetuses				Litter weight	Single fetus		Hemoglobin	
		Total implants	Live	Stunted <sup>2</sup>	Resorbed		Weight	Length	Mother	Fetus
Controls 7-16th day 37 Rats	a 36.13 b $\pm 12.6$ c	352 $9.5 \pm 3.3$	349 9.4 $\pm 3.4$ 99.15 $\pm 0.49$	— — —	3 $0.08 \pm 0.82$ $0.85 \pm 0.94$	44.6 $\pm 5.2$	4.7 $\pm 1.3$	4.4 $\pm 0.79$	12.6	
Controls 5-14 day, Water, 30 Rats	a 36.7 b $\pm 11.3$ c	282 $9.4 \pm 2.36$	277 9.25 $\pm 2.7$ 98.3 $\pm 2.3$	— — —	5 $0.16 \pm 0.37$ $1.7 \pm 2.4$	38.8 $\pm 12.0$	4.2 $\pm 0.54$	4.0 $\pm 0.18$	12.6	10.9
$3 \times 30$ mgm./kg. prior to mating, 20 Rats	a 29.5 b $\pm 23.4$ c	164 8.2	150 7.5 91.5	10 $0.5 \pm 1.15$ $6.7 \pm 3.8$	13 $0.65 \pm 1.66$ $7.9 \pm 2.1$	32.6 $\pm 59.9$ $\pm 18.8$	4.35	4.0 $\pm 0.48$	13.95	
2.5 mgm./kg. 5-14 days, 22 Rats	a 29.7 b $\pm 12.5$ c	207 9.4	190 8.8 86.5	12 $0.54 \pm 1.2$ $6.3 \pm 1.76$	16 $0.73 \pm 2.1$ $7.7 \pm 1.85$	35.0 $\pm 12.8$	4.06	3.3 $\pm 0.4$	12.7	
$1 \times 30$ mgm./kg. 4th day, 25 Rats	a 37.2 b $\pm 12.5$ c	223 8.9	214 8.6 88.3	17 $0.68 \pm 1.97$ $7.9 \pm 3.4$	9 $0.36 \pm 1.11$ $4.0 \pm 1.32$	34.9 $\pm 13.8$	4.1	4.1 $\pm 0.31$	12.4	12.4
$2 \times 30$ mgm./kg. 4 & 5 day, <sup>3</sup> 22 Rats	a 17.9 b $\pm 9.1$ c	245 11.3	215 9.8 96.4	96 $4.6 \pm 4.41$ $44.6 \pm 3.39$	30 $1.36 \pm 1.53$ $12.2 \pm 6.6$	6.4 $\pm 2.2$	0.65	1.8 $\pm 0.23$	13.7	
$2 \times 5$ mgm./kg. 7th day, 24 Rats	a 30.7 b $\pm 11.3$ c	208 8.7	171 7.1 82.4	13 $0.54 \pm 1.67$ $7.6 \pm 1.14$	37 $1.5 \pm 1.97$ $17.6 \pm 2.64$	28.1 $\pm 9.5$	3.8	3.7 $\pm 0.28$		



TABLE 1 (continued)

5 mgm./kg. 7 & 8 day, 22 Rats	a b c	26.8 ±10.1	197 9.0	110 5.0 56	17 0.77 ± 15.4 ±	2.7 7.24 ±	87 4.0 ± 44.0 ±	23.1 ±11.5	3.8 3.6 ±0.14	13.9	10.6
5 mgm./kg. 7, 8, 9 days, 22 Rats	a b c	23.0 ±14.5	187 8.5	88 4.0 47	39 1.8 ± 44.3 ±	3.24 5.18 ±	99 4.5 ± 53.0 ±	21.7 ±9.7	3.5 3.6 ±0.52	13.8	11.9
10 mgm./kg. 7 & 8 day, 21 Rats	a b c	14.3 ±9.0	191 9.1	18 0.86 9.4	4 0.19 ± 22.1 ±	4 0.68 9.8	173 8.2 ± 90.5 ±	13.2 ±11.2	3.7 4.1 ±0.57	15.9	
10 mgm./kg. 6-MP & Leu- covorin 7 & 8 day, 25 Rats	a b c	16.3 ±10.8	229 9.2	22 0.88 9.6	11 0.44 ± 50.0 ±	1.0 10.6	207 8.3 ± 90.5 ±	8.4 ±3.6	3.5 3.6 ±0.28	15.4	11.3
20 mgm./kg. 7 & 8 day, 22 Rats	a b c	16.0 ±2.47	194 8.8	63 2.9 32.5	13 0.59 ± 20.6 ±	1.56 5.1	131 5.95 ± 67.5 ±	31.9 ±16.5	4.0 3.9 ±0.42	13.4	
30 mgm./kg. 7 & 8 day, 41 Rats	a b c	10.3 ±4.64	381 9.3	23 0.56 6.0	10 0.24 ± 43.5 ±	0.23 7.66	258 8.7 ± 94.0 ±	10.8 ±7.5	3.3 3.3 ±0.46	14.4	
60 mgm./kg. 7 & 8 day, 17 Rats	a b c	5.9 ±3.27	151 8.8				151 8.8 100.0				
5 mgm./kg. 12 & 13 day, 23 Rats	a b c	33.15 ±12.7	183 8.0	175 7.6 95.5	3 0.13 ± 1.64 ±	0.34 0.96	8 0.35 ± 4.37 ±	34.7 ±14.6	4.4 3.9 ±0.29	13.4	10.0
10 mgm./kg. 12 & 13 day, 24 Rats	a b c	33.2 ±8.4	201 8.4	189 7.9 94	7 0.29 ± 3.7 ±	0.24 1.37	12 0.50 ± 5.97 ±	31.7 ±10.8	3.8 3.9 ±0.94	13.1	9.8

TABLE 1 (concluded)

		Mother rats percentage weight gain <sup>1</sup> 1/21	Fetuses				Litter weight	Single fetus		Hemoglobin	
			Total implants	Live	Stunted <sup>2</sup>	Resorbed		Weight	Length	Mother	Fetus
20 mgm./kg. 12 & 13 day, 25 Rats	a	35.3	216	206	22	9	31.6	3.8	3.8	13.3	11.2
	b	±8.7	8.67	8.4	0.88 ±	0.36 ±	±8.7	±0.02			
	c			95.4	10.6 ±	4.2 ±					
30 mgm./kg. 12 & 13 day, 28 Rats	a	29.9	253	229	28	14	30.6	3.74	3.95	12.9	9.45
	b	±7.9	9.04	8.2	1.0 ±	0.5 ±	±3.48		±0.29		
	c			90.5	12.4 ±	5.53 ±					

(1) Per cent weight gain of mother rats during gestation period.

(2) Of live fetuses.

(3) Sacrificed on 16th day of gestation.

(a) Total number.

(b) Single rat.

(c) Percentage.

the brain of the living fetuses; in short, no gross malformation, apart from general stunting, was noted in any fetus. However, minor defects detectable on serial sections and reconstruction would, of course, be missed with the technique employed.

*Treated animals.* (1) Rats treated with three doses of 30 mgm. kg. prior to mating showed toxic effects, disturbed oestrus, and a delay of mating for 10 days. Significant lesions of the litter consisted in the appearance of a few stunted fetuses and a small increase in the number of resorbed fetuses.

(2) One or two doses of 30 mgm. kg., given on the fourth or fourth and fifth days of fetal life, resulted in the appearance of stunted fetuses—amounting to 44 per cent in rats receiving two doses plus a slight increase in the number of resorbed fetuses.

(3) The effect of 6-MP at the time of *implantation* (seventh and eighth days) was as follows. Two doses of 60 or 30 mgm./kg. were toxic to the mothers, and so many rats died of diarrhea and anemia that a second group receiving the 30 mgm. kg. dose was used. The implantations, especially in rats receiving the 60 mgm. kg. doses, were abortive. The ova implanted but no proper placenta developed. The site of implantation, however, was clearly recognizable by the large stalk of blood vessels in the uterine wall, by a superficial mucosal defect in the decidua, and sometimes by the presence of membranes. With doses of 5 to 20 mgm. kg., placental development of varying size was noted at all implantation sites, whether fetuses were present or not. The results showed that doses of 10 to 60 mgm. kg. prevented normal weight gain of the mother rats during gestation, resulted in the stunting of many living fetuses, and produced fetal death with resorption in a very high percentage of all fetuses of the litters. The litter weight of the surviving litters with fewer than normal numbers alive was reduced, but the average weight and length of the few surviving fetuses were almost normal except for the rats receiving the 30 mgm./kg. doses.

A group of rats treated with the 10 mgm./kg. dose on the seventh and eighth day, but receiving in addition 10 mgm./kg. of Leucovorin prior to each 6-MP dosage and also on the ninth day, showed no evidence of protection against the effect of 6-MP; the percentage of resorbed fetuses being identical with the group not treated with Leucovorin.

The group of rats receiving two doses of 5 mgm./kg. showed, by comparison to the controls, a large number of resorbed and stunted fetuses. Fifty-six per cent of all the implantations developed fetuses, of which 15 per cent were stunted in growth. The others were quite normal in size and weight. Following this observation, it was attempted to decide experimentally whether individual variations in the time of implantation, or individual susceptibility to the drug, was responsible for the striking differences in reaction to the drug within the same litter. For this reason, two 5-mgm./kg. doses were given on the seventh day at 12-hour intervals, while another group of rats received single 5-mgm./kg. doses on the seventh, eighth, and ninth day of gestation. It was intended, with this dose application, to cover the entire possible implantation period. The number of resorptions fell from 44 to 17 per cent when 6 MP was given on the seventh day only, and rose only to 53 per cent when given on the



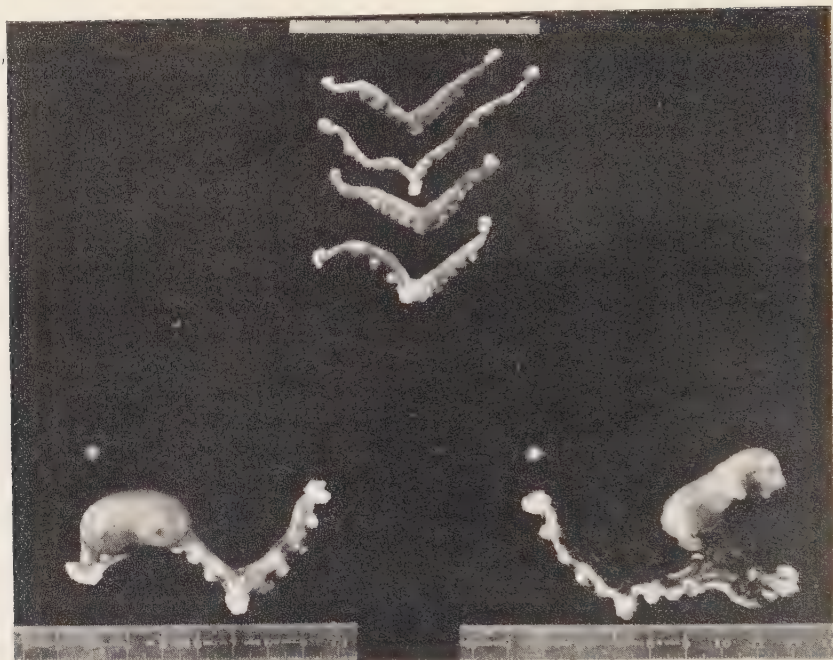


FIGURE 1. Uteri of rats treated with 6-mercaptopurine on the seventh and eighth day and sacrificed on the 21st day of gestation. In the center, uteri with abortive implantation sites from rats treated with two 30 mgm./kg. doses. At the bottom, the uterus of a rat with a single surviving fetus treated with two 10 mgm./kg. doses.

seventh, eighth, and ninth days. The results listed in TABLE 1 clearly demonstrate that the individual young fetus varies in its susceptibility to the drug even at the time of implantation to such a degree that it can survive its litter mates. Experiments with a 2.5-mgm./kg. dose given 10 times from the fifth through the fourteenth day (or a total of 25 mgm./kg.) of pregnancy had little effect on the fetuses. A small percentage of the fetuses were stunted, and the number of resorbed fetuses showed only a slightly significant increase to 7.7 g ( $\pm 1.85$ )—a finding in sharp contrast to the effect of  $2 \times 10$  mgm./kg., or the total dose of 20 mgm. kg. at the time of implantation, where 90 per cent of all fetuses had died.

(4) The effect of 6-MP on the 12th and 13th day of gestation was investigated with doses of 5 to 30 mgm. kg. Apart from stunting a few fetuses, doses of 5, 10, 20, and 30 mgm./kg. had only a slight effect on the litters. The number of resorptions, while increased, did not vary significantly statistically from the control groups. The results showed that the older fetus was not very susceptible to the drug.

### *Summary*

- (1) Fetal death and stunting of some of the surviving fetuses were induced by 6-MP when given orally in nontoxic doses to the mother rats.
- (2) The most sensitive period of the fetus to the drug was the time of im-

plantation, and 24 hours thereafter, or the seventh and eighth day of gestation. During this period, two doses of 5 mgm./kg. induced death and resorption of half of all fetuses and two doses of 10 mgm./kg. induced 90 per cent fetal resorptions. Even larger doses had but little effect when given on the fourth and fifth or on the 12th and 13th day of fetal life. Leucovorin given concurrently with 6-MP on the seventh and eighth day had no protective action against the drug. No accumulative effect of 10 doses of 2.5 mgm./kg. was noted when given from the fifth to the fourteenth day of gestation. Gross malformations were not noted in any fetus—not even in single survivors of otherwise destroyed litters.

(3) Rats treated prior to mating with 3 doses of 30 mgm./kg. produced litters with a small increase in numbers of stunted and resorbed fetuses suggesting an adverse effect, possibly an incorporation of the drug in the developing ovum.

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# EFFECTS OF 6-MERCAPTOPURINE ON EXPERIMENTAL TUMORS IN TISSUE CULTURE\*

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Sarcoma cell division is differentially inhibited by 6-mercaptopurine in combined cultures of mouse sarcoma 180 and mouse embryo skin.<sup>1</sup> Inhibition of mitosis has now been sought in tissue cultures of a variety of other tumors and normal tissues of mouse and man. In addition, biochemical agents antagonistic to this action of 6-mercaptopurine have been sought.

## *Procedure*

Studies on mitosis were made in tissue cultures grown in plasma clots on coverslips in roller tubes, as reported previously.<sup>1</sup> The medium was a conventional one of serum, embryo extract, and balanced salt solution. One day after the cultures were planted, they were dosed by replacement of one tenth of the one milliliter of fluid medium per roller tube with a saline solution of the dosing agent. After 24 hours of exposure to the agent, the cultures were fixed in alcohol-acetic acid 3:1 and stained with the Feulgen reaction and light green. Counts of mitotic figures and pyknotic or degenerating nuclei were then made in 1000 nuclei in the zone of outgrowth in each of several cultures for a given treatment. These data are entered in the tables as means of the several counts at a given concentration.

The author is indebted to Doctor K. Sugiura of the Sloan-Kettering Institute for the mouse tumors T241, Ma 387, carcinoma 1025, Ehrlich carcinoma, and Miyono adenocarcinoma. The author is indebted to Doctor Helene Toolan of the Sloan-Kettering Institute for rat-carried transplants of the human sarcoma, Toolan's HS No. 1, in its 33rd passage, and the human epidermoid carcinoma, Toolan's HEP No. 3, in its eighth passage.

Mouse tumors other than sarcoma 180 were dosed two days after being planted, because of their slower outgrowth. The human tumors were dosed when they had developed equivalent outgrowths. This treatment was 24 hours after planting for HEP No. 3, and 72 hours after planting for HS No. 1. Exposure to the agent was for 24 hours in all cases.

## *Results*

The differential mitotic inhibition of sarcoma 180 cells in tissue culture by 6-mercaptopurine‡ is illustrated in TABLE 1. These data on sarcoma 180 and embryo mouse-skin cells were presented at a recent conference of the New York Academy of Sciences<sup>1</sup> and are to be considered in relation to the report by Clarke *et al.* (1953) that 6-mercaptopurine causes regression of sarcoma 180

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† With the technical assistance of Miss Marilyn Margolis, Miss Virginia Babcock and Mrs. Marilyn Clarke Slauterback.

‡ The 6-mercaptopurine was kindly supplied by Doctor George H. Hitchings of the Wellcome Research Laboratories.

TABLE 1

MEAN INCIDENCE PER 1000 NUCLEI OF MITOTIC (MIT.) AND DEGENERATING (DEG.) NUCLEI IN CULTURES OF MOUSE TUMORS, EMBRYO SKIN, AND NEWBORN HEART TREATED FOR ONE DAY WITH 6-MERCAPTOPYRINE

6-MP	S180		Skin		Heart		T241		Ma387		1025		Ehrlich		Miyono	
	Mit.	Deg.	Mit.	Deg.	Mit.	Deg.	Mit.	Deg.	Mit.	Deg.	Mit.	Deg.	Mit.	Deg.	Mit.	Deg.
0 mM	87	8	83	19	67	2	20	137	9	24	11	36	59	8	55	11
4.0	5	341	63	15	24	4	8	353	0	393	10	16	12	70	16	73
2.0	7	205	55	27	44	1	10	316	1	183	5	29	22	42	27	30
1.0	10	128	58	19	34	2	10	189	9	49	7	22	14	31	22	21
0.5	18	85	86	6	49	3	18	81	6	17	6	42	30	33	36	13
0.1	80	30	93	6	65	2	26	120	10	12	7	56	29	30	41	22

in mice in a considerable proportion of cases. In the 6-mercaptopurine concentrations that severely curtail cell division in sarcoma 180 cultures, mitotic incidence in mouse embryonic skin cultures is only slightly affected.

Newborn mouse-heart cultures show an intermediate effect (TABLE 1). Mitosis is suppressed to one half or one third the control incidence by high concentrations of 6-mercaptopurine. There is no appreciable increase in the proportion of degenerating nuclei in treated heart cultures.

The other five mouse tumors studied show intermediate or low sensitivity to 6-mercaptopurine (TABLE 1). The two sarcomas, T241 and Ma387, suffer reduced mitotic incidence and a considerable increase in pyknosis under higher concentrations of 6-mercaptopurine. Carcinoma 1025 is unaffected by the agent in culture. Both the Ehrlich carcinomas and the Miyono adenocarcinoma cultures show reduced mitotic incidence under higher concentrations of 6-mercaptopurine, but they have less nuclear degeneration than the sarcoma cultures. Results of two separate experiments with each tumor are in good agreement.

Sugiura (1953) found that these tumors treated in mice show the following order of increasing sensitivity to 6-mercaptopurine (the first two are refractory *in vivo*): T241 = Ma387 < Miyono < Ehrlich < 1025 < S180. The tissue-culture results are not in complete accord, notably in the placing of carcinoma 1025. T241 and Ma387 do show mitotic suppression and considerable nuclear death with 6-mercaptopurine *in vitro* despite their resistance *in vivo*. It could be argued that the mitotic suppression in cultures of the Ehrlich carcinoma and the Miyono adenocarcinoma is proportionately of the same order of magnitude as that in T241 and Ma387 cultures, but the greater absolute number of degenerating nuclei in treated cultures of the two sarcomas suggests that T241 and Ma387 be ranked as more sensitive *in vitro* than the Ehrlich and Miyono tumors. This ranking would not agree with Sugiura's findings *in vivo*.

Toolan's human sarcoma (HS) No. 1 is more sensitive to 6-mercaptopurine than Toolan's human epidermoid carcinoma (HEP) No. 3 *in vitro* (TABLE 2). Mitotic incidence is reduced in HS No. 1 cultures exposed to 4.0 down through 0.5 mM 6-mercaptopurine, but in HEP No. 3 cultures, mitosis is partly suppressed only by 4.0 and 2.0 mM 6-mercaptopurine.



TABLE 2

MEAN INCIDENCE PER 1000 NUCLEI OF MITOTIC AND DEGENERATING NUCLEI IN CULTURES OF TWO HUMAN TUMORS TREATED WITH 6-MERCAPTOPYRINE FOR ONE DAY

6-MP	HS No. 1		HEP No. 3	
	Mit.	Deg.	Mit.	Deg.
0 mM	75	5	94	37
4.0	16	147	52	24
2.0	33	44	48	51
1.0	47	34	97	36
0.5	47	37	96	29
0.1	69	19	94	7

TABLE 3

LACK OF INFLUENCE OF LIVER EXPLANTS ON 6-MERCAPTOPYRINE EFFECTS  
Mean incidence per 1000 nuclei of mitotic and degenerating nuclei in cultures of sarcoma 180 treated for one day

6-MP	S180 alone		S180 + liver	
	Mit.	Deg.	Mit.	Deg.
0 mM	59	42	69	35
4.0	4	264	6	241
2.0	14	240	10	284
1.0	17	237	24	231
0.5	39	91	32	193
0.1	56	63	76	31

Experiments on the mechanism of action of 6-mercaptopurine were made with coverslip cultures of sarcoma 180 and sometimes of mouse-embryo skin.

Because fluorophenylalanine effects on sarcoma 180 cultures were accentuated or changed in the presence of explants of newborn mouse liver,<sup>2</sup> similar experiments were carried out with 6-mercaptopurine. The results (TABLE 3) indicate that the effectiveness of 6-mercaptopurine on sarcoma 180 cultures is unchanged in the presence of mouse-liver explants.

The physiological purines were tested for antagonism to the antimitotic properties of 6-mercaptopurine (TABLE 4). At equimolar concentration, hypoxanthine gives moderate protection, guanine and adenine slight or equivocal protection, and xanthine none against 1.0 mM 6-mercaptopurine. Such effectiveness as any of these purines has at 1.0 mM is not expressed at 0.1 mM.

In antagonism studies with nucleosides of the physiological purines (TABLE 5), inosine is most effective in partly countering the mitotic inhibition of 6-mercaptopurine. Adenosine and 2'-desoxyadenosine\* have somewhat less activity, and xanthosine little, if any. In other experiments, guanosine had about the same activity as adenosine.

A series of adenosine phosphates was also tested (TABLE 6). Adenylic acid

\* The 2'-desoxyadenosine was kindly provided by Doctor Donald W. Visser of the University of Southern California.

TABLE 4

## PHYSIOLOGICAL PURINE BLOCKING OF 6-MERCAPTOPURINE

Mean incidence per 1000 nuclei of mitotic and degenerating nuclei in cultures of sarcoma 180 treated for one day

6-MP	S180	
	Mit.	Deg.
0 mM	82	33
1.0	13	225
1.0 + adenine 1.0 mM	25	250
1.0 + guanine 1.0 mM	31	203
1.0 + hypoxanthine 1.0 mM	36	79
1.0 + xanthine 1.0 mM	6	295

TABLE 5

## NUCLEOSIDE BLOCKING OF 6-MERCAPTOPURINE

Mean incidence per 1000 nuclei of mitotic and degenerating nuclei in cultures of sarcoma 180 treated for one day

6-MP	S180	
	Mit.	Deg.
0 mM	82	33
1.0	13	225
1.0 + inosine 1.0 mM	47	136
1.0 + xanthosine 1.0 mM	16	119
0	87	25
1.0	16	219
1.0 + adenosine 1.0 mM	29	128
1.0 + 2'-desoxyadenosine 1.0 mM	35	112

*b* (adenosine-3'-phosphate) is most effective against 6-mercaptopurine for both sarcoma 180 and embryonic skin cells. Adenylic acid *a* (adenosine-2'-phosphate) and AMP (adenosine-5'-phosphate) are less effective. ATP is ineffective with sarcoma 180 cultures but effective with skin cultures.

With all of these physiological purines and purine derivatives at 1 mM, the greatest effect any of them shows against 6-mercaptopurine for sarcoma 180 cultures is in bringing the mitotic incidence back up to about half of the control value.

Much better results were given in a single, as yet unrepeatable experiment with coenzyme A, carried out at the suggestion of Doctor Donald A. Clarke of the Sloan-Kettering Institute. TABLE 7 presents results.

The coenzyme A preparation, at a concentration of 0.5 mgm. per ml. of medium, maintains mitotic activity at normal levels in sarcoma cultures simultaneously treated with 1.0 mM 6-mercaptopurine, *i.e.* 1.0 micromole/ml. of medium. A dose of 0.05 mgm. of the coenzyme A preparation is slightly less effective. Neither concentration of coenzyme A shows much effect against 4.0 mM 6-mercaptopurine. Coenzyme A alone stimulates proliferation of sarcoma 180 cells *in vitro*. Similar but less dramatic results are obtained with embryonic skin cultures.

TABLE 6

## ANTAGONISM BETWEEN ADENOSINE PHOSPHATES AND 6-MERCAPTOPURINE

Mean incidence per 1000 nuclei of mitotic and degenerating nuclei in cultures of mouse sarcoma 180 and embryo skin treated for one day

6-MP	S180		Skin	
	Mit.	Deg.	Mit.	Deg.
0	101	22	73	22
1.0 mM	6	258	49	20
1.0 + adenylic <i>a</i> 1.0 mM	24	224	61	35
1.0 + adenylic <i>b</i> 1.0 mM	45	193	90	26
1.0 + AMP 1.0 mM	23	279	41	159
0	87	25	94	9
1.0 mM	16	219	56	51
1.0 + ATP 1.0 mM	11	233	91	38

TABLE 7

## COENZYME A BLOCKING OF 6-MERCAPTOPURINE

Mean incidence per 1000 nuclei of mitotic and degenerating nuclei in cultures treated for one day

6-MP	"CoA"	S180		Skin	
		Mit.	Deg.	Mit.	Deg.
0	0	111	26	75	20
4.0 mM	0	4	480	51	20
4.0	0.5 mg/ml	23	301	73	25
4.0	0.05	9	430	40	26
1.0	0	28	205	53	26
1.0	0.5	109	38	65	31
1.0	0.05	96	101	46	27
0	0.5	160	26	97	17
0	0.05	131	12	85	21

The coenzyme A preparation was a commercial one of approximately 30 per cent purity. Hence the 0.5 mgm. dose contained about 150 micrograms or about 0.2 micromoles coenzyme A, and the 0.05 mgm. dose contained about 0.02 micromoles coenzyme A. The other 70 per cent of the preparation may well have also contained metabolites antagonistic to 6-mercaptapurine. In the 0.05 mgm. dose, the extra 70 per cent might have amounted to about one fifth of a micromole of some purine, for example. As it happens, our previous experiments indicate that this amount of physiological purine could not account for the entire extent of the blocking of 1.0 mM 6-mercaptapurine.

In another part of this experiment, insulin was tested in combination with 6-mercaptapurine (TABLE 8). Although insulin is essentially without effect on the reaction of sarcoma 180 cultures to 6-mercaptapurine, it does affect the reaction of embryonic skin cells. The slight drop in mitotic incidence in embryonic skin cultures treated with 4.0 or 1.0 mM 6-mercaptapurine is considerably accentuated by concomitant treatment with 1.0 or 0.25 unit insulin per ml. of medium. Treatment with insulin alone does not diminish mitotic incidence in embryonic mouse-skin cultures.

TABLE 8

EFFECT OF INSULIN ON RESPONSE OF MOUSE TISSUE CULTURES TO 6-MERCAPTOPYRINE  
Mean incidence per 1000 nuclei of mitotic and degenerating nuclei in cultures treated for one day

6-MP	Insulin	S180		Skin	
		Mit.	Deg.	Mit.	Deg.
0 mM	0	111	26	75	20
4.0	0	4	480	51	20
4.0	1.0 unit/ml.	5	395	19	41
4.0	0.25	2	507	18	92
1.0	0	28	205	53	26
1.0	1.0	12	248	23	39
1.0	0.25	21	212	48	20
0	1.0	100	43	84	18
0	0.25	97	28	69	25

### Discussion

It may be concluded from the foregoing that 6-mercaptopurine is capable of suppressing cell division to variable extent in a number of cell strains in tissue culture. In some neoplastic cell strains the mitotic inhibition is more pronounced than in the several normal tissues tested.

The mitotic suppression caused by 1.0 mM 6-mercaptopurine in sarcoma 180 cultures can be relieved in part by a variety of physiological purines, their nucleosides, and their nucleotides in equimolar concentration. The material most effective in counteracting the mitotic inhibition is a preparation of coenzyme A. From the 30 per cent concentration of coenzyme A in the preparation, it follows that the active material may be something other than coenzyme A, or it may be a fortunate combination of growth factors, perhaps including coenzyme A. If the active material be coenzyme A itself, two molecules of coenzyme A are seen capable of completely blocking the mitotic inhibition caused by 100 molecules of 6-mercaptopurine, when the latter is applied at a concentration of 1.0 mM. If the active material be coenzyme A, it follows that 6-mercaptopurine may be interfering in 2-carbon transfers or in the energy metabolism of the citric acid cycle in our tissue cultures.

The insulin results may lead to similar conclusions, for Paul and Leslie (1954) have shown that insulin, in stimulating growth, energy production, and the synthesis of protein and nucleic acid in chick-embryo tissue cultures, increases glucose utilization and causes a profound drop of the pyruvic acid concentration in the medium. It may be that 6-mercaptopurine has a more pronounced effect when substrate for the citric acid cycle is deficient. However, it may also be that the increased nucleic acid synthesis brought about by insulin leads to a greater incorporation of 6-mercaptopurine into polynucleotides.

### Summary

- (1) Mitosis of sarcoma cells in combination tissue cultures of mouse sarcoma 180 and embryonic skin is differentially inhibited by 6-mercaptopurine.
- (2) Mitosis in tissue cultures of a number of other mouse tumors and two human tumors is suppressed by 6-mercaptopurine to a variable extent.



(3) The effect of 6-mercaptopurine on sarcoma 180 cultures is not influenced by the presence of explants of newborn mouse liver.

(4) Several physiological purines, nucleosides, and nucleotides partly block the mitotic inhibition caused by 6-mercaptopurine in sarcoma 180 cultures.

(5) A coenzyme A preparation is most effective in relieving the mitotic suppression caused by 6-mercaptopurine.

(6) Insulin, which does not affect mitotic incidence when used alone, greatly increases the susceptibility of embryo mouse-skin cultures to mitotic suppression by 6-mercaptopurine.

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# EFFECTS OF 6-MERCAPTOPURINE AND ANALOGS ON EXPERIMENTAL TUMORS\*

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6-Mercaptopurine (6-MP) initially stimulated interest in cancer chemotherapy with the demonstration that it inhibited the growth of the Crocker mouse sarcoma 180 (S-180). An extension of this observation by Doctors Kanematsu Sugiura and George Tarnowski disclosed that 6-MP could significantly inhibit the growth of nine other mouse tumors and two different rat tumors. These results are summarized in TABLES 1 and 2. Since the majority of the data included in this presentation have been obtained in studies on S-180, the remainder of this report will be confined to this system.

For the demonstration of the inhibitory actions of 6-MP it was customary to inject daily doses by the intraperitoneal route in the form of finely ground suspensions in 0.5 per cent carboxymethyl cellulose† in 0.85 per cent NaCl ("CMC suspension"). Another method of preparation consisted of dissolving 6-MP with *ca* 1.5 molar equivalents of NaOH and then precipitating in dilute solution, by the addition of HCl, to adjust the pH to *ca* 7.0; isotonic NaCl was employed as the diluent ("saline suspension"). The daily dose was contained in 0.5 ml. of either preparation. The agent was also employed as a supplement to the diet.

Female Swiss albino mice (Millerton Industries, Inc.) weighing 18 to 22 gm. were employed throughout the studies. Uniform pieces of S-180, weighing *ca.* 5 mgm. (wet), were introduced subcutaneously by trocar in the right axillary region; therapy was initiated either 24 or 96 hours after implantation and continued for seven successive days. Twenty-four hours after the last dose, the tumors were measured through the skin with calipers. Two diameters, one of which was the greater and the other a diameter perpendicular to it, were measured for each tumor and used in the calculation of average diameters.

Studies with other purine analogs were conducted in a similar manner, except that 2,6-diaminopurine (2,6-DAP) was used as the lactate and injected at body temperature in solution in isotonic NaCl, and that 6-chloropurine and purine, being water-soluble, were employed dissolved in distilled water.

During treatment with 6-MP initiated 24 hours after implantation, the growth of S-180 was moderately inhibited, as seen in TABLE 3. When pieces of tumor from mice receiving such a course of therapy were implanted by trocar into normal mice, the majority of implants failed to grow. This result was in

\* These studies were supported by an institutional grant from the American Cancer Society. Supplies of 6-mercaptopurine employed in these studies have been made available by Doctor George H. Hitchings of the Wellcome Research Laboratories, Tuckahoe, N. Y. The 2,6-diaminopurine, thioguanine, 6-methylpurine, and 8-azaguanine were provided by the same source. Supplies of 2-azaguanine were provided by Doctor Karl Pfister of Merck and Company, Rahway, N. J. Purine and 6-chloropurine, the synthesis and characterization of which will be described,<sup>3</sup> were provided by Doctor Aaron Beech of the Sloan-Kettering Institute, New York, N. Y. The authors wish to express their sincere gratitude for the interest and generosity of these collaborators.

† High viscosity cellulose gum, Hercules Powder Co.

TABLE I  
6-MERCAPTOPURINE ON MOUSE TUMORS\*

Tumor	Inhibition
Sarcoma 180.....	+
Bashford carcinoma 63.....	+
Ehrlich carcinoma.....	+
Carcinoma 1025.....	+
Epidermoid carcinoma.....	+
Adenocarcinoma E 0771.....	+
Adenocarcinoma 755.....	+
Adrenocarcinoma RC.....	+
Miyono adenocarcinoma.....	+
Ridgway osteogenic sarcoma.....	+
Sarcoma T-241.....	Slight
Sarcoma MA-387.....	Slight
Wagner osteogenic sarcoma.....	Slight
Patterson lymphosarcoma.....	0
Mecca lymphosarcoma.....	0
Gardner lymphosarcoma.....	0
Harding-Passey melanoma.....	0
Andervont hepatoma.....	0

\* CMC suspension of 6-MP injected I.P., 30 mgm./kg./day for 7 successive days; therapy initiated 24 hours after tumor implantation. Data from Sugiura<sup>4</sup> and Tarnowski.<sup>5</sup>

TABLE 2  
6-MERCAPTOPURINE ON RAT TUMORS\*

Tumors	Inhibition
Flexner-Jobling carcinoma.....	+
Sarcoma R-39.....	+
Walker Carcino-sarcoma.....	Slight
Murphy-Sturm lymphosarcoma.....	0
Jensen sarcoma.....	0

\* CMC suspension of 6-MP injected I.P., 30 mgm./kg./day for 7 successive days; therapy initiated 24 hours after tumor implantation. Data from Sugiura.<sup>4</sup>

contrast to the behavior of untreated S-180, which almost invariably "takes" by this method of implantation.<sup>7</sup> Furthermore, when treated mice were held for extended observation, a prolongation of survival time occurred and a significant number of individuals recovered from the tumor (FIGURE 1). This reaction was in contrast with the behavior of untreated S-180, which is highly lethal to its host.

Treatment with 6-MP initiated 96 hours after implantation, though it failed to inhibit tumor growth during the course of therapy and increased host intoxication, did promote eventual recovery from tumor of approximately 50 per cent of mice given a 7-day course of therapy of 50 mgm./kg./day.

The nature of tumor disappearance in mice treated with 6-MP was studied, and a fundamental difference observed between treated and untreated animals. In untreated mice a varying but low incidence of recovery from S-180 occurs. This spontaneous process is characteristically one of tumor necrosis and ulceration through the skin followed by *extrusion* of the tissue. By contrast, *extrusion* of tumor tissue was rarely seen in groups of mice receiving 6-MP. The

TABLE 3  
INHIBITION OF GROWTH OF SARCOMA 180 AND TOXICITY TO HOST WITH  
6-MERCAPTOPYRINE\*

Dose mgm./kg./day	No. of mice	% Mortality during therapy	At End of Therapy		
			Average weight change gm.	Average tumor diameter	
				mm.	± S.D.
100	30	33	-2.5	4.1	±1.4
0	30	0	±0	10.8	±1.2
75	30	10	-1.5	5.8	±1.7
0	30	3	-0.5	11.5	±1.1
50	30	0	-0.5	7.1	±2.2
0	30	0	+2.0	10.5	±1.9
25	50	0	+0.5	8.4	±1.5
0	5	0	+1.0	11.0	±1.0

\* Therapy initiated 24 hours after tumor implantation and continued for seven successive days; the 25 mgm. group was a saline suspension; all others, CMC suspension. These data from another study (6).

majority of mice recovering from S-180 after therapy with 6-MP showed *re-sorption* of the tumor mass. It was customary to observe such animals for at least four weeks from the time the tumor was no longer palpable; recurrence of growth was not observed. Whether metastatic lesions might subsequently have developed is a question which was not explored, but this possibility must be considered for either treated or untreated recoveries.

Tumors were removed at 24-hour intervals from mice receiving a daily dose of 100 mgm. kg. of 6-MP; the specimens were fixed in Zenker-formol and stained with hematoxylin and eosin. The progressive histological changes have been described in detail elsewhere,<sup>6</sup> and only the major disturbances will be summarized. The tumors were largely composed of giant cells, and multi-nucleated forms were prominent. Nuclei were large and possessed varied, bizarre, chromatin patterns. There was hyperchromasia, some pyknosis, and both cytoplasmic and nuclear vacuolization (FIGURES 2 and 3). According to the criteria usually employed by pathologists, the majority of cells were neither dead nor dying. On the other hand, the data summarized above suggest that the majority of these cells were incapable of further growth. Furthermore, recent *in vitro* studies by Doctor Henry Mihich\* have indicated a simultaneous depression of the  $QO_2$  and  $QCO_2$  to the extent of 50 per cent or more in tumor slices obtained from treated animals. These observations suggest that the metabolic capacities of treated tumors are in the direction of dying tissues. At present, these contrasting observations seem paradoxical.

The fact that all transplants were not equally susceptible to destruction by 6-MP and also the fact that a small number of treated tumors were found to "take" when reimplanted into untreated hosts suggested that S-180 might contain a small population of resistant cells, or might actually develop resistance

\* Visiting Research Fellow, from the University of Milan.



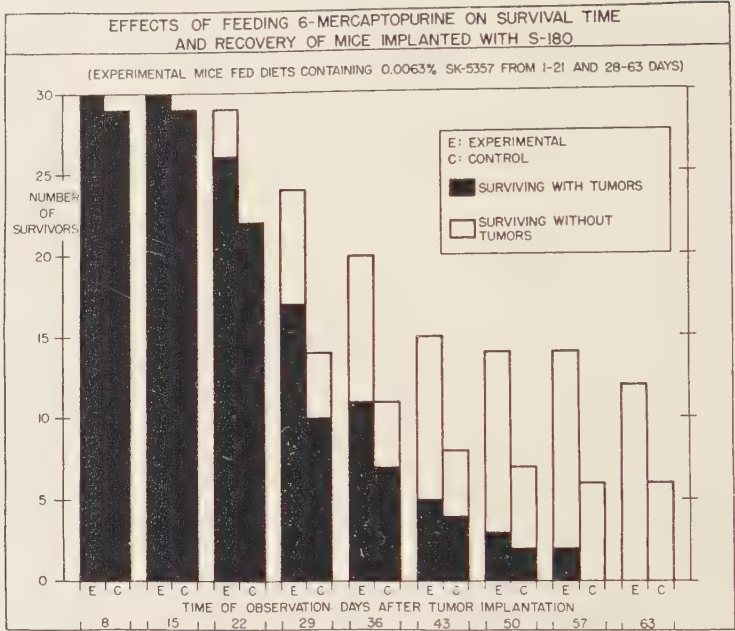


FIGURE 1. Increase in recovery from S-180 following exposure to 6-MP in the diet (ground Purina Chow). Both experimental and control mice were permitted food and water *ad libitum*. Recovery from tumor in treated mice was resorptive; in control mice, extrusion of necrotic tumor mass through ulcerated skin. (SK-5357 is 6-MP.) Data from another study (6).

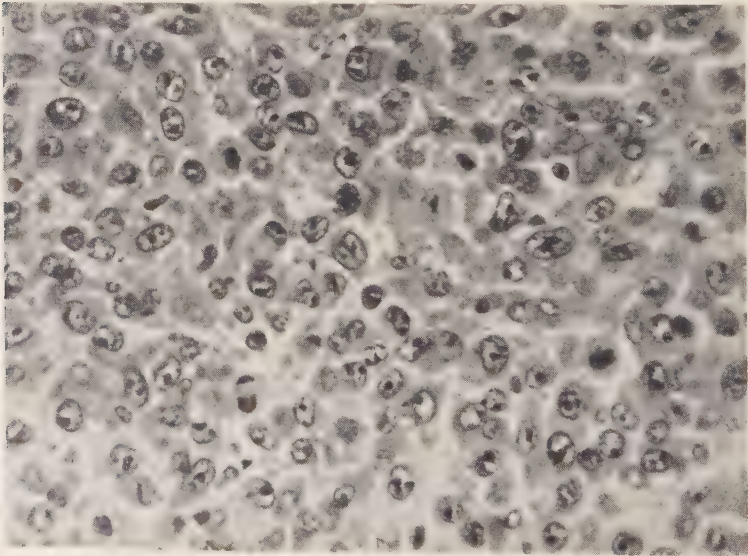


FIGURE 2. Normal, untreated sarcoma 180 showing tumor cells with but slight variation in general appearance. 400 X. From a previous study (6).

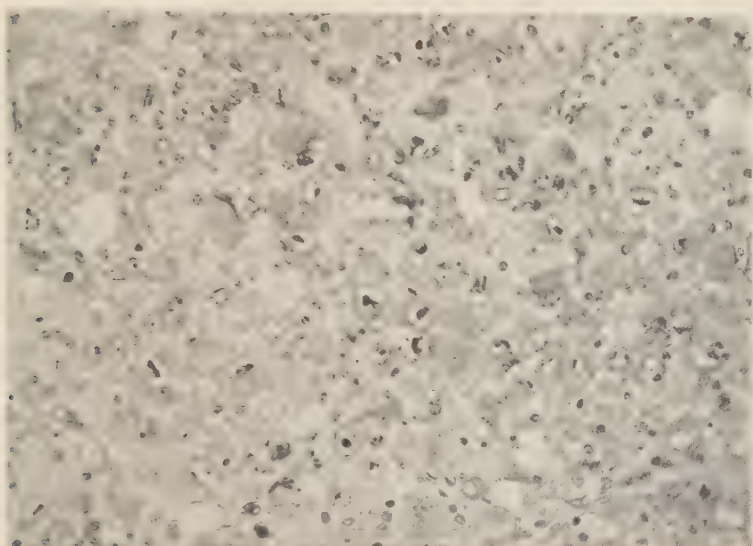


FIGURE 3. S-180 from a mouse treated with 6-MP (100 mgm./kg./day for seven consecutive days). Considerable cytoplasmic vacuolization, irregularity of nuclei, cytoplasm and nuclei, and variation in shape of cells. 400 X. From a previous study (6).

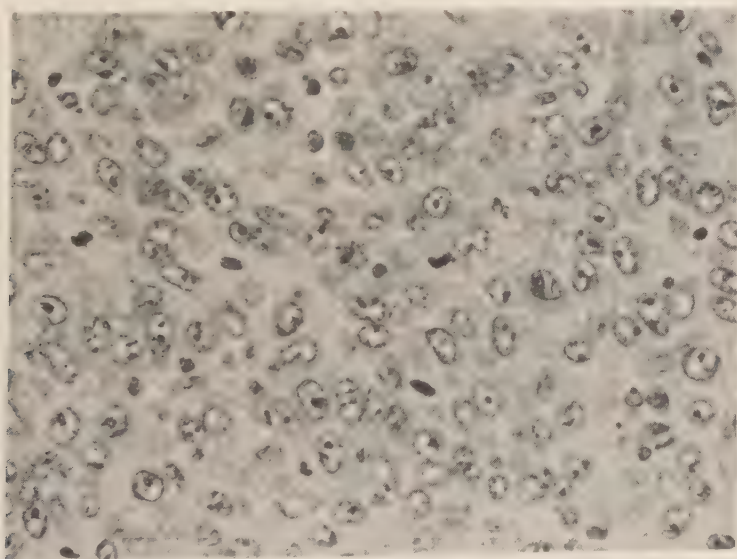


FIGURE 4. Untreated S-180 resistant to 6-MP. Histologic appearance almost identical to tumor in FIGURE 3. Normal, untreated, except for slight cytoplasmic vacuolization. 400 X. From a previous study (6).

during therapy. A treated tumor, which grew following implantation into a normal host, was accordingly treated through successive transplant generations with low doses of 6-MP (25 mgm./kg./day). After two generations, its growth was found resistant to treatment with higher doses (100 mgm./kg./day)

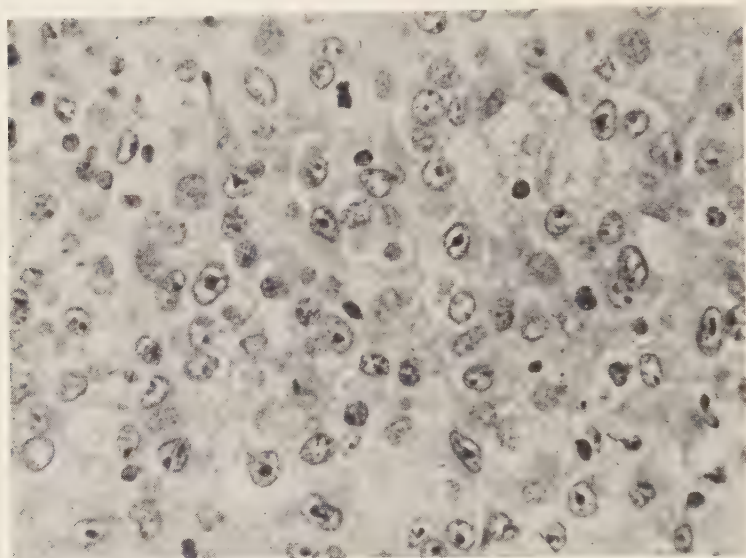


FIGURE 5. S-180 resistant to 6-MP following seven days of therapy (100 mgm./kg./day). Similar in appearance to tumor in FIGURES 2 and 4. In this particular field the cytoplasmic vacuolization is more pronounced than usual. 400 X. From a previous study (6).

Furthermore, this resistant tumor, after courses of high-dose therapy with 6-MP, exhibited 100 per cent "takes" when implanted in normal mice. Cytological study of the resistant tumor revealed that it differed from sensitive S-180 by the presence of cytoplasmic vacuolization. Treatment did not alter its appearance (FIGURES 4 and 5). Tumors from this resistant line have been maintained through 28 transplant generations without further exposure to 6-MP and have retained the characteristic of resistance.

The results obtained with 6-MP in the S-180 system stimulated similar studies with other substituted purines. The inability of guanine, 8-azaguanine, adenine, 2-azaadenine, and 2,6-DAP to inhibit growth of S-180 was confirmed. Active purines included, in addition to 6-MP, 2-amino-6-mercaptopurine (thioguanine), 6-chloropurine, 6-methylpurine, and purine. FIGURE 6 illustrates the dose-response relationship among three of these agents: thioguanine, 6-MP, and chloropurine. It may be seen, at the 50 per cent inhibition point, that the ratio of effectiveness among the compounds was, respectively, 1:23:238. These three inhibitors appeared to be similar in action as judged by the following criteria: the slopes of the dose-response curves were similar; treated tumors failed to grow when reimplanted into normal hosts; cytologically, the tumor lesions induced by the agents were similar, differing only in degree of alteration; the 6-MP resistant tumor was cross-resistant to both thioguanine and 6-chloropurine; and recoveries following therapy with 6-chloropurine occurred by resorption as previously described for 6-MP.

Thioguanine was an exception to the last criterion. Although, in some instances, resorption occurred, the majority of recoveries were the result of ne-

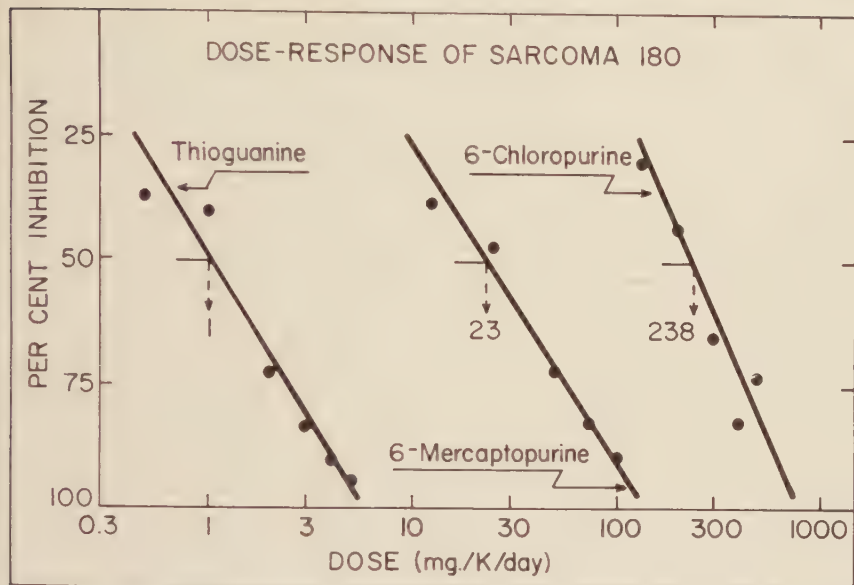


FIGURE 6. Relationship of response of S-180 to doses of various inhibitors. Tumor sizes were determined 24 hours after a last dose of a 7-day course of therapy. The relative potency of the three analogs, as tumor inhibitors, is illustrated; the similarity in the slope of the individual curves suggests a common mechanism.

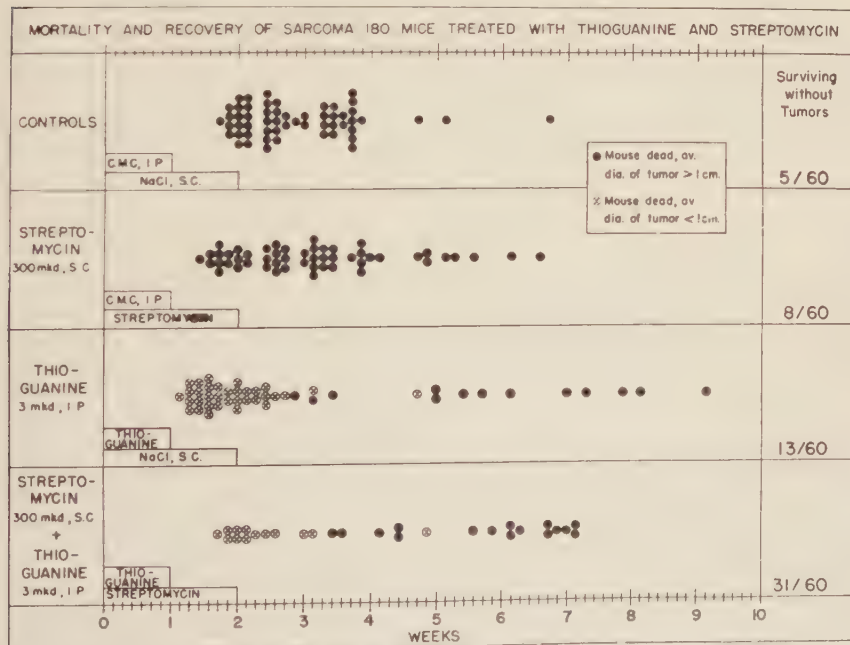


FIGURE 7. Influence of antibiotic therapy on survival of mice receiving the tumor-damaging, granulocytopenia-inducing agent, thioguanine.



crotic dissolution of inhibited tumors. The necrosis of these tumors may be associated with the systemic toxicity of thioguanine. Thioguanine, in the usual inhibitory doses, was a compound toxic to mice; deaths usually occurred several days after cessation of therapy. To seek the cause of this intoxication, mice with S-180 were sacrificed at the end of treatment for total autopsies, bone-marrow smears, and peripheral blood counts. The only significant lesions observed were reduction in red pulp of the spleen and nearly complete depletion of bone marrow. These lesions were reflected in the peripheral blood by marked granulocytopenia. It appeared that septicemia, incident to agranulocytosis, could have been responsible for the delayed deaths caused by treatment and could possibly account for the large incidence of tumor necrosis in survivors.

To explore this possibility, mice were concomitantly treated with streptomycin and thioguanine. FIGURE 7 summarizes the results of two such experiments conducted at different times of the year. In these experiments, streptomycin significantly decreased the lethality of thioguanine and enhanced the rate of recovery from tumor. It is to be emphasized that in mice given both streptomycin and thioguanine, tumor recoveries were largely by resorption.

Purine and 6-methylpurine, for reasons at present obscure, are paradoxical members in the series. Purine is erratic as a tumor inhibitor; its dose-response curve is in the range of that for 6-chloropurine, but the slope varies. Recoveries may occur following the resorption pattern; however, it is difficult to anticipate when these effects will appear. It is possible that such variations may be associated with systemic intoxication of the host, such as the kidney lesion caused by purine, which is described in the companion report by Philips *et al.*

The tumor inhibitory action of 6-methylpurine is delayed; a week after completion of therapy, inhibition of growth becomes evident, but not at the time a course of therapy is concluded. Systemic toxicity has a role in limiting the use of this agent; doses which are inhibitory ultimately lead to death of the animal.

### Conclusion

The adenine analog, 6-mercaptapurine, effectively inhibits growth of sarcoma 180, resulting in prolongation of survival time of the host and, in a significant number of individuals, recovery from the tumor. 6-MP also inhibits growth of a number of other, but not all, experimental rodent tumors. 6-MP induces alterations in S-180 which result in loss of viability of the otherwise readily transplantable tumor; these changes appear to be responsible for the resorption of tumor, which results in recovery of treated individuals. S-180 is capable of developing into a tumor resistant to both the inhibitory and oncolytic effects of 6-MP; it is reasonable to deduce that this phenomenon is responsible for the therapeutic failures recorded. Related compounds, thioguanine, 6-chloropurine, 6-methylpurine, and purine, also inhibit growth of S-180 but differ from 6-MP in certain respects.

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# RESPONSE OF ACUTE LYMPHOCYTIC LEUKEMIAS TO THE PURINE ANTAGONIST 6-MERCAPTOPURINE

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Transformations to resistance or dependence have been obtained in leukemic cells of the mouse by the use of several antimetabolites: folic acid analogs<sup>1,2,4</sup> and the triazolopyrimidine analog of guanine, 8-azaguanine.<sup>5</sup> These transformations have been shown to be stable, irreversible, and heritable, and have been produced readily in several transplantable lines of lymphocytic leukemia.<sup>6</sup> Experimental findings strongly indicate that the variant lines produced by the use of these antimetabolites arise by mutation. The role of antimetabolite is merely that of a selective agent.<sup>7</sup>

Preliminary evidence which suggests that purine analogs act as antagonists of nucleic acid synthesis has encouraged investigation of the role of these compounds in the inhibition of cancerous growths. The adenine analog, 6-mercaptopurine, has been shown to act as a purine antagonist in the metabolism of *Lactobacillus casei*.<sup>9</sup> It has also been shown to be a unique inhibitor of sarcoma 180<sup>10</sup> and of certain mammary adenocarcinomas.<sup>11</sup> Limited clinical trials of this compound in advanced leukemia of children have been encouraging.<sup>12</sup>

Definite, regular, and reproducible inhibition of growth, in some cases striking, of leukemic cells has been obtained in several transplantable lines of acute lymphocytic leukemia of the mouse. No increase in survival time of mice bearing other transplantable acute lymphocytic leukemias could be obtained. See TABLE 1 for a comparison of effects of antifolics and antipurines on several transplantable leukemias. In leukemia L1210,<sup>13</sup> moderate increases in survival time were observed and are described here. Preliminary data have been published.<sup>8</sup> Following the methods used previously in obtaining transformations to folic acid antagonists<sup>4</sup> and 8-azaguanine,<sup>5</sup> two resistant sublines of leukemia L1210 have been developed using 6-mercaptopurine as the selective agent. Some of the pertinent characteristics of one of these L1210 resistant leukemic lines will be considered.

Transplant generations 202 to 270 of the sensitive subline of leukemia L1210 have been used as control material for the present study. Beginning with the 202nd transfer, a standard dose of leukemic cells ( $8 \times 10^5$  cells) in Lockes' solution was inoculated subcutaneously in the right axillary region of 18–20 g DBA/2 strain mice. These mice subsequently received daily injections, beginning at 24 hours, of the adenine analog, 6-mercaptopurine.\* The compound was dissolved in 0.1 N NaOH and then diluted with sterile saline so that the constant volume injected subcutaneously was 0.025 cc. per gram of body weight. Daily injections were continued until palpable growths sufficient for transfer were obtained. During the first three transfers, the total

\* This compound as well as other antipurines furnished by Doctor George Hitchings of the Wellcome Research Laboratories, Tuckahoe, N. Y. A-Methopterin and 8-azaguanine were supplied by Lederle Laboratories Division, American Cyanamid Company.

TABLE 1  
RESPONSE OF SEVERAL TRANSPLANTABLE ACUTE LYMPHOCYTIC LEUKEMIAS TO  
ANTIFOLIC AND ANTIPURINE COMPOUNDS

	Line of leukemia in strain of mouse						
	L1210 DBA	4946 AKR	L3054 C58	L5178 DBA	L5397 DBA	L5208 C58	L5769 AKR
A-Methopterin .....	++	++	+	++	±	+	+
8-Azaguanine .....	+	—	—	++	++	—	+
6-Mercaptopurine .....	+	—	—	+++	++	—	+
Thioguanine .....	+	—	—	+++	?	?	?
2,6-Diaminopurine .....	±	—	—	++	?	?	?

+ = 100%; ++ = 200%; +++ = 300% increase in survival time; ± = questionable response; — = no response; ? = untested.

TABLE 2  
RESISTANCE IN LEUKEMIA L1210 (DBA 2 MICE) FOLLOWING CONTINUOUS TREATMENT  
WITH THE ADENINE ANALOG 6-MERCAPTOPYRINE

Transfer generations	No. mice	Time transplanted in days	Dosage daily	Mgm./kg.) total	Tumor weight (mgm.) at 8 days
L1210-6-mercaptopurine-resistant					
G-1 to 3 <sup>1</sup>	3 each	9-11	37.5	262.5	none
G-4	5	8	75	525	280.4
"	5	8	None		420
G-5 to 7	3 each	8	75	525	carried only
G-8	5	8	75	525	81
"	5	8	None		45
G-9 to 12	3 each	8	75	525	carried only
G-13	10	8	75	525	200
"	10	8	None		251.8
G-14 to 39	5 each	7 to 8	75	525	approx. 500
L1210 sensitive					
G-206	8	8	75	525	0
	8	8	None		450.9
G-210	8	8	75	525	0
	8	8	None		496.3
G-212	8	8	75	525	0
	8	8	None		590.2

<sup>1</sup> Transfer generation G-1 was derived from transfer generation G-202 of the L1210 sensitive line; thus, transfer generation G-206 is the control transfer for G-4 of the resistant subline.

dosage of analog was 262.5 mgm./kg. (37.5 mgm./kg.  $\times$  7 days) and subsequently the total dosage was 525 mgm./kg. (75 mgm./kg.  $\times$  7). Transfers of leukemic cells were done at 9 to 11 days during the first four transfer generations, and later at seven and eight days as larger subcutaneous growths appeared in animals receiving the near-MTD dosage. The difference in response to 6-mercaptopurine in the transfer line grown only in mice receiving the analog and in the control (sensitive) line may be seen in TABLE 2. Very little difference in weights of the subcutaneous tumor grown in mice with and without



analog were observed in the line being developed for resistance, whereas complete inhibition of growth of leukemic cells, at eight days, was observed in the sensitive control line at comparable transfer generations.

The 6-mercaptopurine-resistant (6-MR) subline has now been carried through 70 consecutive transfers in DBA/2 mice receiving the near-MTD of this analog, 525 mgm./kg. (75 mgm./kg.  $\times$  7). Sizeable tumor growths of nearly 500 mgm. are always obtained in the presence or absence of the analog, indicating the development of resistance. The dependence phenomenon characteristic of other transfer lines has not been observed, although only one other variant subline (also resistant) has been developed in this laboratory, using this compound. That the variant cells described here exhibit resistance to 6-mercaptopurine is shown also in experiments using leukemic death, following intraperitoneal transfers of the standard dose of leukemic cells as the criterion. The mean survival time of mice bearing the sensitive subline of L1210 was  $8.0 \pm 0.06$  days, whereas an increase of survival of 87.0 per cent, to  $14.8 \pm 0.18$  days was obtained using 6-mercaptopurine within the total dosage range of 250-1200 mgm./kg. In contrast, identical survival times were obtained in mice bearing the 6-mercaptopurine-resistant cells, with and without the analog (TABLE 3).

TABLE 3  
EFFECTS OF PURINE ANALOGS AND AMETHOPTERIN ON SURVIVAL TIME OF MICE BEARING (I) SENSITIVE LEUKEMIA L1210 AND (II) 6-MERCAPTOPYRINE-RESISTANT 6-MR LEUKEMIA L1210

Compound	No. separate expts.	Total no. mice	Dosage (mgm./kg.)		Mean survival time in days (range)	% Increase in survival
			Individual	Total		
(I) L1210-sensitive <sup>2</sup>						
Controls.....	20	161 <sup>1</sup>	None		8.0 ( 7-9 ) <sup>3</sup>	—
6-Mercaptopurine...	17	122	50-125	250-1000	14.8 (10-22) <sup>4</sup>	87.0
8-Azaguanine.....	6	56	75	450-600	12.4 (10-20)	60.0
8-Azaxanthine.....	5	48	75-113	450-800	8.1 ( 7-10)	—
2,6-Diaminopurine	3	24	75	450-600	8.0 ( 7-10)	—
Thioguanine.....	4	26	7.5-10.0	20-30	13.7 (12-15)	73.5
A-Methopterin.....	6	51	3	24	17.1 (14-23)	117.0
(II) L1210-6-MR						
Controls.....	5	100	None		9.9 ( 9-11)	—
6-Mercaptopurine...	5	82	75	525-675	10.1 ( 9-11)	—
8-Azaguanine.....	4	34	75	600	9.6 ( 9-11)	—
8-Azaxanthine.....	5	37	75	600	9.5 ( 9-13)	—
2,6-Diaminopurine	4	34	75	600	9.7 ( 9-11)	—
Thioguanine.....	5	42	7.5	30	9.7 ( 8-11)	—
A-Methopterin.....	4	34	3	24	27.4 (22-38)	175.0

<sup>1</sup> CDF<sub>1</sub> mice ♀ BALB/c  $\times$  DBA/2 F<sub>1</sub> hybrids and strain DBA/2 used. No differences in survival times were noted between DBA and CDF<sub>1</sub> mice, nor between sexes.

<sup>2</sup> Comparable transfer generations were used for these data; for L1210 sensitive, transfers 202 to 270 and for the transformed, 6-mercaptopurine resistant line, transfers 10(210) to 70 (270).

<sup>3</sup> Standard errors are given in the text.

<sup>4</sup> Mean age at death from leukemia of mice receiving total dosage of 6-mercaptopurine at the 250 mgm./kg. level was 13.2 days; 300-450 mgm./kg.—15.3 days; 525-600 mgm./kg.—15.3 days and 750-1000 mgm./kg.—14.2 days. See text.

Significant and reproducible increases in survival time in DBA/2 or CDF<sub>1</sub> mice bearing the sensitive, control leukemia L1210 were obtained at dosage levels of 250 mgm./kg. (50 mgm./kg.  $\times$  5 days) to 1000 mgm./kg. (125 mgm./kg.  $\times$  8 days). Slight losses in body weight, never exceeding 1.5 gm. were obtained only at dosage levels of 600-1000 mgm./kg. Within the dosage range of 250 to 600 mgm./kg. regular increases in body weight were obtained. No deaths attributable to the drug were found at the highest dosage level used. The effects obtained at the higher levels were within the same range as those obtained at the 300 to 600 mgm./kg. level. At 200 mgm./kg. (50 mgm./kg.  $\times$  4) however, only a 50 per cent increase in survival time was obtained.

The possibility was considered that resistance developed to one purine-antagonist might extend to other purine antagonists and possibly to folic acid antagonists. Of the compounds used, the triazolopyrimidine analog, 8-azaguanine, has been shown to have leukemia-inhibiting properties in certain leukemias, especially in the lymphocytic leukemia L1210 used in this study. Thioguanine has also been shown to increase survival time of L1210 leukemic mice effectively and regularly, whereas 8-azaxanthine (resulting from deamination of 8-azaguanine) and 2,6-diaminopurine are ineffective. The comparative effects of these compounds, as well as of the folic acid antagonist, A-Methopterin (4-amino-N<sup>10</sup>-methyl PGA) are shown in TABLE 3. Resistance to 6-mercaptopurine is accompanied by resistance to all other purine and pyrimidine antagonists studied, including two tested recently, purine and chloropurine. This effect is unlike the resistance phenomenon in *L. casei*, in which resistance to this compound was not accompanied by resistance to other adenine antagonists, particularly 2,6-diaminopurine, 8-azaadenine and purine, and 8-azaguanine, a guanine antagonist.<sup>14</sup>

Dosage levels of 6-mercaptopurine as high as 600 mgm./kg. were without effect on survival time of DBA/2 or CDF<sub>1</sub> mice bearing an 8-azaguanine-resistant line of L1210, indicating again cross-resistance; also, the analog provided for near-optimal growth, as determined either by weight of localized lymphomatous tissue or survival time of an 8-azaguanine dependent line of this leukemia.<sup>15</sup>

It has been shown in tests employing *L. casei* that 6-mercaptopurine inhibition of growth is not reversible with folic acid (pteroylglutamic acid).<sup>14</sup> Further evidence that this analog is not an "antifolic" is shown in the sensitivity of the L1210 6MR subline to 4-amino-N<sup>10</sup>-methyl PGA. A more striking inhibition of leukemic cell growth by this folic acid antagonist is seen in mice bearing intraperitoneal transplants of the 6-mercaptopurine-resistant subline than in mice bearing the control L1210-S subline, particularly at higher levels (3 mgm./kg.  $\times$  8 days). An increase in survival time of nearly 200 per cent, from  $9.9 \pm 0.10$  days to  $27.4 \pm 0.90$  days was obtained, whereas an increase from  $8.0 \pm 0.6$  to  $17.1 \pm 0.69$  was shown among controls. It remains to be determined whether this increased sensitivity to a folic acid antagonist results from a significantly increased requirement for folic acid, which is found to be characteristic of a strain of *Lactobacillus casei* resistant to 6-mercaptopurine.<sup>11</sup> It is interesting to note that two other transformations in leukemia L1210, resistant to 8-azaguanine (L1210-8AG-R) and dependent upon 8-azaguanine (L1210-

TABLE 4  
INCREASED SENSITIVITY TO A-METHOPTERIN OF LEUKEMIC LINES TRANSFORMED THROUGH THE USE OF ANTIPIRINES

Compound	L1210(S)		L1210-8AG-D		L1210-8AG-R		L1210-6MR	
	No.	Survival	No.	Survival	No.	Survival	No.	Survival
None.....	145	8.0 ± 0.06	68	15.8 ± 0.45	42	10.2 ± 0.11	42	9.9 ± 0.10
8-Azaguanine.....	56	12.4 ± 0.20	74	12.1 ± 0.23	42	9.9 ± 0.09	—	—
6-Mercaptopurine.....	86	14.8 ± 0.18	—	—	—	—	42	10.1 ± 0.10
A-Methopterin.....	51	17.1 ± 0.69 (117%)	81	63.1 ± 3.2 <sup>1</sup> (300%)	32	36.9 ± 1.5 <sup>2</sup> (269%)	34	27.4 ± 0.90 (175%)

Dosage schedules: 8-azaguanine, 75 mgm./kg. X 8 days subcutaneously; 6-mercaptopurine, 75 mgm./kg. X 7 days subcutaneously; A-Methopterin, 3 mgm./kg. X 8 days, intraperitoneally. Treatment begun 48 hours after inoculation of  $8 \times 10^5$  leukemic cells intraperitoneally.

<sup>1</sup> 39 mice (48.2%) negative at 120 days.

<sup>2</sup> 5 mice (16%) negative at 120 days; survival time determined at 90 days for all negative animals.

TABLE 5  
PARTIAL REVERSAL OF 6-MERCAPTOPURINE ACTIVITY BY HYPOXANTHINE

	Leukemic deaths—days									
	6	7	8	9	10	11	12	13	14	15
Controls.....	—	7	15	1	—	—	—	—	—	—
6-Mercaptopurine.....	—	—	—	—	1	8	12	2	2	—
6-Mercaptopurine + Hypoxanthine.....	—	—	5	10	8	17	1	—	—	—
Hypoxanthine.....	—	8	12	—	—	—	—	—	—	—

Dosage: 6 mercaptopurine = 50 mgm./kg.  $\times$  4; hypoxanthine 300-500 mgm./kg.  $\times$  4. Hypoxanthine given one hour prior to 6-mercaptopurine.

SAG-D) are also accompanied by even more striking sensitivity to the folic analog, 4-amino- $N^{10}$  methyl PGA, resulting in increases in survival of leukemic mice over the controls to 300 per cent<sup>15</sup> (TABLE 4). On the other hand, resistant and dependent leukemic cells developed by the use of 4-amino- $N^{10}$  methyl PGA (A-Methopterin) have not shown an increased sensitivity to the purine antagonists.

Extensive attempts to reverse the antileukemic activity of 6-mercaptopurine in the L1210 sensitive line by natural purines have been relatively unsuccessful. Guanine (25 and 50 mgm./kg.  $\times$  4), adenine (50, 75, 100, and 150 mgm./kg.  $\times$  4), xanthine (100 and 300 mgm./kg.  $\times$  4) and hypoxanthine (100, 300, and 500 mgm./kg.  $\times$  4) were given intraperitoneally every other day, beginning at 24 hours, following inoculations of leukemic cells, and one hour prior to injections of 50 mgm./kg. of 6-mercaptopurine.

It is interesting to note that, only through the use of hypoxanthine, was any consistent reversal observed. In five of six experiments, nearly 50 per cent reversal of antileukemic activity was obtained, especially at dosage levels of hypoxanthine of 300 to 500 mgm./kg.  $\times$  4. A sample reversal experiment is shown in TABLE 5. Unlike the results with the ribosides and ribotides of adenine and guanine in reversing the antileukemic activity of 8-azaguanine,<sup>14</sup> reversal of 6-mercaptopurine activity under the dosage schedule employed in these experiments, was most difficult. Only occasionally, with adenosine, was partial reversal obtained. In *L. casei*, on the other hand, growth inhibition by this antipurine is prevented competitively, and apparently with ease, by any of the four physiologic purine bases.<sup>13</sup>

Potential of the effect of 6-mercaptopurine is obtained when this analog is given to mice bearing L1210 sensitive leukemia in conjunction with A-Methopterin. The mean increase in survival of DBA/2 mice given A-Methopterin (3 mgm./kg.  $\times$  4-8 days) plus 6-mercaptopurine (75 mgm./kg.  $\times$  7 days) was 185 per cent, whereas in this series the purine analog alone increased life expectancy 70 per cent, and the folic analog 80 per cent. This combination of compounds was not as effective, however, as A-Methopterin and 8-azaguanine given in combination.<sup>16</sup>

No morphologic changes have been observed in the sensitive line of leukemia following exposure to this adenine analog, or in the resistant line developed by continuous exposure *in vivo*.



*Summary.* The adenine analog, 6-mercaptopurine, has been shown to inhibit leukemic cell growth of leukemia L1210 and several other acute lymphocytic leukemias in a definite, regular, and reproducible manner. Lower dosage levels of this compound appear to be as effective as higher (near-MTD) levels as measured by the criterion of leukemic death.

A resistant subline of leukemia L1210 has been developed by consecutive passage of leukemic cells in DBA/2 strain mice receiving daily injections of 6-mercaptopurine. Resistant leukemic cells grow optimally either in the presence or absence of the antagonist.

Resistance to 6-mercaptopurine is accompanied by resistance to all other purine analogs tested: 8-azaguanine, 8-azaxanthine, 2,6-diaminopurine, thio-guanine, purine, and chloropurine, some of which are moderate antileukemic agents in the sensitive line of this transplantable leukemia.

An increased sensitivity to the folic acid antagonist, 4-amino-N<sup>10</sup> methyl PGA (A-Methopterin) is a characteristic of the resistant variant as well as of two other variant lines developed through the use of 8-azaguanine.

Potentiation of antileukemic activity was obtained using the folic analog, A-Methopterin and 6-mercaptopurine in combination, given simultaneously.

Attempts to reverse the antileukemic activity of 6-mercaptopurine by the physiologic purines adenine, guanine, xanthine, and hypoxanthine have been relatively unsuccessful. Only in the case of hypoxanthine have definite and reproducible reversals been obtained. Among the ribosides and ribotides of adenine and guanine, only adenosine has given partial reversals.

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# STUDIES ON THE TOXICITY AND ANTILEUKEMIC ACTION OF 6-MERCAPTOPURINE IN MICE

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It has been observed that 6-mercaptopurine acts as a purine antagonist for *Lactobacillus casei*.<sup>1,2</sup> The free purines adenine, guanine, xanthine, and hypoxanthine blocked the inhibitory activity of 6-mercaptopurine for this organism in a competitive manner: 6-mercaptopurine has also been demonstrated to have an inhibitory effect on the growth of experimental tumors and human neoplasia.<sup>3-7</sup> However, as in the case of other known antineoplastic agents, the toxicity of the drug for the host is a limiting factor in its employment in the treatment of neoplasia.

Although the triad of host-parasite-drug has long been recognized and treated in quantitative fashion in infection chemotherapy,<sup>8-10</sup> there has been relatively little emphasis on the host-tumor-drug relationship in tumor chemotherapy. Emphasis has been placed, in our laboratory, on the development of qualitative experimental procedures for the study of the host-tumor-drug relationship. It was felt that such procedures could provide a more firm basis for evaluation of drug effectiveness and could provide additional means for study of the mode of action of drugs. Employing citrovorum factor, folic acid, and aminopterin, experimental procedures were employed which indicate that, in the mouse, the analysis of dose-response relationships may provide a basis for inhibition analysis.<sup>11,12</sup> In addition, a microbiological assay procedure was developed which provides a quantitative description of the antineoplastic specificity of action of a drug in terms of its relative effect against the tumor and the host.<sup>13-15</sup> This procedure permits the comparison of the relative anti-tumor specificity of action of different treatments with the same drug, as well as of different drugs. Employing these procedures, studies were undertaken on the interrelationships of host, tumor, and drug, employing 6-mercaptopurine in mice.†

## I. Dose-Mortality Relationships in Mice Without Tumor

If 6-mercaptopurine acts as an antimetabolite in the whole animal, it should be possible to reduce its toxicity by appropriate administration of metabolite. Attempts in this direction have been successful.<sup>16</sup>

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‡ The 6-mercaptopurine was kindly provided by Doctor George Hitchling, of the Wellcome Research Laboratories. Guanylic acid, adenylic acid (yeast), 2' adenylic acid, 3' adenylic acid, adenosine, thymidine, adenine sulfate, and adenosine triphosphate were obtained from Schwarz Laboratories, Inc.; 5' adenylic acid (M-B Den) was from Ernst Bischoff Co. Guanosine, inosine, deoxyribose nucleic acid, sodium deoxyribonucleate, thymine, uracil, adenine, xanthine, uridylic acid, and cytidylic acid were from Nutritional Biochemical Co. Diphosphopyridine nucleotide reported in TABLE 1 and 3 was from Sigma Chemical Company. Diphosphopyridine nucleotide reported in TABLE 4 was from Pubst Laboratories Division of Pabst Brewing Co. Ribonucleic acid (acid nucleic) was from Fischer Scientific Co. Guanine and hypoxanthine were from Bio. Laboratories; L-amino-ε-imidazole carboxamide was from the California Institute of Biochemical Research. Citrovorum factor (leucovorin) was from the Calco Chemical Division of the American Cyanamid Co.

In the first phase of this study, the compounds tested were administered to mice one hour prior to the 6-mercaptopurine. The lethal toxicity of 6-mercaptopurine was diminished by the prior administration of each of the following: guanylic acid, adenylic acid (yeast), 2' adenylic acid, 3' adenylic acid, 5' adenylic acid, adenosine, guanosine, inosine, diphosphopyridine nucleotide, adenosinetriphosphate, ribosenucleic acid, desoxyribosenucleic acid, sodium desoxyribosenucleate, and thymidine (TABLE 1). The weight loss generally associated with 6-mercaptopurine toxicity was reduced when the animals were protected by administration of metabolite. The following compounds gave little or no protection: adenine, guanine, xanthine, hypoxanthine, uridylic acid, cytidylic acid, thymine, uracil, citrovorum factor, and 4-amino-5-imidazole carboxamide (TABLE 1).

On concomitant administration, as well as on prior administration of metabolite, the toxicity of 6-mercaptopurine is dependent upon the relative doses of inhibitor and metabolite employed. As the dose of 6-mercaptopurine was increased, there was a progressive increase in the mortality response (TABLES 2, 3). As the dose of effective metabolite was increased, there was a decrease in the mortality response (TABLES 1-3).

The extent of protection may be limited by toxic action of the metabolite at high doses. In the lower dose range of adenylic acid (0-100 mgm./kg.), there was a progressive decrease in toxicity of 6-mercaptopurine as the dose of adenylic acid was increased (TABLE 2). In this range, as in the case of Aminopterin plus citrovorum factor<sup>11, 12</sup> the toxicity of 6-mercaptopurine is apparently a function of the ratio of the inhibitor to the metabolite added plus a constant representing the amount of endogenous protection present in the normal animal expressed in terms of metabolite. However, as the adenylic acid dosage was increased to 800 and 1600 mgm./kg.; these doses themselves exerted relatively little toxicity, the protective action appearing to diminish (TABLE 2). The data suggest that, in the presence of 6-mercaptopurine, the toxicity of adenylic acid is in some way increased. The increased toxicity fails to show up unless the adenylic acid is at a near-toxic level. We accordingly have a relationship between two drugs which is antagonistic in one direction, but potentiating in the other.

Relations such as the above may exist between 6-mercaptopurine and other drugs. When such a relationship does exist, it makes difficult the identification of drugs which are protective against 6-mercaptopurine, when only one dose level is used. It may well be that drugs reported here and elsewhere as nonprotective would prove otherwise if they were subjected to more thorough investigation.

A preliminary comparison was made of the relative effectiveness of various metabolites in protecting against 6-mercaptopurine toxicity on simultaneous administration of the drugs. On a molar basis, adenylic acid appeared to be the most effective; diphosphopyridine nucleotide and adenosinetriphosphate were next in effectiveness; ribosenucleic acid, guanylic acid, and thymidine were less effective; adenine was the least effective (TABLE 3). Approximate estimates of the inhibition index for these metabolites, expressed as the slope relating 6-mercaptopurine LD50 to metabolite administered, on a molar basis, are listed

TABLE 1

EFFECT OF PRIOR ADMINISTRATION OF METABOLITES ON THE LETHAL TOXICITY  
OF 6-MERCAPTUPURINE IN MICE\*

Experiment	Metabolite	Dose mgm./kg.	6-Mercaptopurine dose mgm./kg.		
			0	500	750
			Dead/total		
1	Guanylic Acid	1500	2/10	0/10	
		None		9/10	
2	Adenylic Acid (yeast)	1000	0/6	0/6	
	2' Adenylic Acid	1000	0/6	0/6	
	3' Adenylic Acid	1000	0/6	0/6	
	5' Adenylic Acid	1000	0/6	0/6	
		None		5/6	
3	Adenylic Acid (yeast)	1000	0/6	0/7	
	Adenosine	1000	0/6	0/7	
	Guanosine	1000	0/6	0/6	
	Diphosphopyridine nucleotide	1500	0/6	0/6	
	" "	1000	0/3	2/6	
		None		6/6	
4	Inosine	1000	0/5	0/5	
	Ribose nucleic Acid	1500	0/5	0/5	
	" " "	1000	0/5	0/5	
	Desoxyribose nucleic Acid	1500	0/5	0/5	
	" " "	1000	0/5	0/5	
	Sodium desoxyribonucleate	1500	0/5	0/5	
	" "	1000	0/5	2/5	
	Thymidine	1000	0/5	0/5	
	Thymine	1000	2/5	5/5	
	Uracil	1000	0/5	2/5	
	Adenine sulphate	1000	2/5	4/5	
		None		7/10	
5	Inosine	1000			0/6
	"	500			4/6
	"	250			6/6
	"	125			6/6
	Adenosinetriphosphate	1000	0/6		1/6
	"	500	0/6		6/6
	"	250			5/6
	"	None			6/6
6	Adenine	1000	3/6	4/5	
	Guanine	1000	0/6	3/6	
	Xanthine	1000	0/3	6/6	
	Hypoxanthine	1000	1/5	3/6	
		None		5/6	
7	Adenine	1000	3/6	4/6	
	"	500	3/6	1/6	
	Guanine	1000	0/6	2/6	
	"	500	0/6	5/6	
	Xanthine	1000	0/6	5/6	
	"	500	0/6	4/6	
	Hypoxanthine	1000	0/6	4/6	
	"	500	0/6	4/6	
		None		8/10	
8	Uridylic Acid	1000	0/6	6/6	
	Cytidylic Acid	1000	0/6	5/6	
		None		4/6	
9	4-Amino-5-imidazole carboxamide	1000	0/6	6/6	
		None		7/8	
10	Citrovorum factor	1000	2/6	5/6	
	" "	200	0/6	6/6	
	Adenylic Acid	1000	0/6	0/6	
		None		10/12	

\* Metabolites were administered one hour prior to 6-mercaptopurine; the latter was injected SC. Metabolites were injected I.P. except in experiment 7 where they were given SC. CDBA hybrid male mice were employed in experiments 1-3 and 6-10. Strain-A male mice were employed in experiments 4 and 5.



TABLE 2  
EFFECT OF CONCOMITANT ADMINISTRATION OF ADENYLIC ACID ON THE TOXICITY OF 6-MERCAPTOPURINE\*

	6-MP Mg/kg	Adenylic acid MG/KG								
		0	12.5	25	50	100	200	400	800	1600
		Number of deaths among 6 treated mice								
<i>Experiment</i> 1	900	6	3	6	0	1	0	1		
	506	6	1	2	0	0	0	0		
	284	3								
	160	0								
	0							0		
<i>Experiment</i> 2	1600					1	4	3	6	6
	1200					2	0	0	1	5
	900	6				0	0	0	0	1
	675	6				0	1	0	0	1
	506	6				1	0	0	0	1
	379	5				0	0	0	0	1
	284	0				0	0	0	1	0
	213	0				0	0	0	0	1
	160	0				0	0	0	0	0
	0	0						0	0	1

\* 6-Mercaptopurine was injected SC. Adenylic Acid was injected I.P. CDBA hybrid male mice.

in TABLE 3. To what extent the relative insolubility of adenine may have contributed to the diminished protection observed is not clear.

The extent of toxicity is also subject to the temporal relationships of administration of metabolite and antimetabolite. The effective metabolites protected well when administered prior to or simultaneously with 6-mercaptopurine. However, the action of 6-mercaptopurine is apparently quite rapid and relatively irreversible. The metabolites tested: adenylic acid, guanylic acid, diphosphopyridine nucleotide, adenosinetriphosphate, ribosenucleic acid, and desoxyribosenucleic acid, showed a marked reduction in their ability to protect when the drugs were withheld for two to four hours following administration of 6-mercaptopurine (TABLE 4). This result is in agreement with the observation of Elion *et al.*, that on administration of labeled 6-mercaptopurine, the peak of activity in most tissues of the mouse occurred in two to three hours.<sup>17</sup>

The analysis of dose-response relationships employing metabolite and anti-metabolite may provide evidence pertaining to the *in vivo* sequence of metabolic transformations. Presumably, the administration of appropriate precursors at requisite doses and correct temporal relationships with respect to the antagonist could afford protection against an antagonist. Folic acid, for example, when administered one hour early, apparently acting as a precursor of citrovorum factor, protected against Aminopterin toxicity in a competitive manner,<sup>11</sup> but afforded no protection on simultaneous administration with the antagonist. Citrovorum factor protected competitively on simultaneous ad-

TABLE 3

COMPARISON OF PROTECTION AFFORDED BY VARIOUS METABOLITES AGAINST THE LETHAL TOXICITY OF 6-MERCAPTOPYRINE\*

	6-MP MG./KG.	Metabolite	Dose mgm./kg.								Inhibition† index approx.	
			0	12.5	25	50	100	200	400	800		1000
			Dead/total									
Experiment 1	750	Adenylic Acid		2/6	2/6	0/6	0/6		0/6		0/6	45
	0										0/6	
	750	Adenosine triphosphate		4/6	6/6	6/6	3/6		0/6	0/6	0/6	8.4
	0								0/6		0/6	
	750	Ribose nucleic acid		6/6	6/6	4/6	4/6		5/6		0/6	3.6
	0								0/6		0/6	
	750	Guanylic acid		6/6	6/6	6/6	5/6		0/6		2/6	2.6
	0										0/6	
	750	Thymidine		5/6	6/6	6/6	6/6		1/6		0/6	1.6
	0										0/6	
Experiment 2	750	Adenine		6/6	6/6	4/6	6/6		4/6		0/6	0.4
	0						0/6		0/6		1/6	
	750	—	27/28									
	500	—	3/6									
	333	—	0/6									
Experiment 2	750	Adenylic acid		5/6	2/6	3/6	0/6	0/6				13.7
	0						0/6					
	750	Diphosphopyridine nucleotide		6/6	5/6	3/6	3/6	0/6	0/6	0/6	0/6	8.7
	0											
	750	—	5/6									
	500	—	2/6									
	333	—	2/6									

\* Metabolites were administered concomitantly with 6-mercaptopurine; the latter was injected SC. Metabolites were injected I.P. CDDBA hybrid male mice.

† Slope relating 6-mercaptopurine LD<sub>50</sub> to metabolite administered on a molar basis.

ministration with Aminopterin. Although no protection against 6-mercaptopurine was observed with 4-amino-5-imidazole carboxamide, the observation of protection with inosine suggests that protection might result from precursors via metabolic transformations involving citrovorum factor. It is of interest that diphosphopyridine nucleotide protects against two types of antagonists in mice. It protects against the lethal toxicity of 6-mercaptopurine and against the lethal toxicity of 3-acetylpyridine.<sup>15</sup> The adenosine moiety is apparently responsible for the protection against 6-mercaptopurine, while the protection against 3-acetylpyridine is attributable to the nicotinamide moiety.

## II. Host-Tumor-Drug Relationships in Mice With Leukemia

The analysis of dose-response relationships has been extended to animals with tumor. A fundamental question in tumor chemotherapy has been: is the antineoplastic specificity of action of a drug, expressed in terms of its relative effect on host and tumor, constant under all circumstances, or may it be

TABLE 4  
TEMPORAL RELATIONSHIP IN THE REVERSAL OF 6-MERCAPTOPURINE TOXICITY BY  
VARIOUS METABOLITES\*

	6-MP mgm./kg.	Metabolite	Time (Hours)	Dose mgm./kg.				
				0	125	250	500	1000
				Dead/total				
<i>Experiment</i> 1	500	Adenylic acid	-1					0/7
	500	" "	0					0/7
	500	" "	+2					3/7
	500	" "	+4					6/7
	0	" "	—					0/20
	500	Gyanylic acid	-1					0/7
	500	" "	0					0/7
	500	" "	+2					7/7
	500	" "	+4					5/7
	0	" "	—					0/20
	500	—	—	9/10				
<i>Experiment</i> 2	750	Adenylic acid	+3		3/6	4/6	2/6	5/6
	0	" "	—					0/6
	750	Adenosine triphos- phate	+3		3/6	6/6	5/6	4/6
	0	" "	—					0/6
	750	Ribose nucleic acid	+3		6/6	6/6	5/6	6/6
	0	" "	—					0/6
	750	Desoxy ribose nucleic acid	+3		6/6	6/6	6/6	5/6
	0	" "	—					0/6
	750	Diphosphopyridine nucleotide	+3		6/6	6/6	5/6	4/6
	0	" "	—					0/6
	750	—	—	14/16				
	500	—	—	3/6				
	333	—	—	1/6				

\* 6-Mercaptopurine was injected SC. Metabolites were injected I.P. CDBA hybrid male mice.

altered? Experiments with Aminopterin, employing leukemia L1210 in mice have shown that the antileukemic specificity of action of Aminopterin is not a fixed property of the drug, but may be altered in accordance with the manner in which the drug is employed. The antileukemic specificity of action of aminopterin was reduced on prior administration of folic acid and on simultaneous administration of citrovorum factor.<sup>14</sup> It was increased on delayed administration of citrovorum factor.<sup>15</sup> Also, the antileukemic specificity of action of Aminopterin was altered by the time at which treatment was given with respect to the growth curve of the tumor and by the schedule of treatment.<sup>19</sup> Several experiments were conducted to determine whether the specificity of action of 6-mercaptopurine with respect to leukemia is a fixed characteristic of the drug, or whether it too may be altered by the manner in which the drug is employed.

The experimental design has been previously described.<sup>14</sup> CDBA hybrid male mice (8 to 12 weeks old) are inoculated in the right thigh with a designated number of tumor cells (leukemia L1210). For each type of treatment, doses are employed to cover, as effectively as possible, the 0-100 percent mortality

range. The experiments are designed to provide a temporal separation of deaths attributable to drug toxicity and deaths attributable to tumor growth. Borderline cases may be separated on the basis of the weight history of the mice and the evidence of local tumor at the site of inoculation.

For each experiment, a set of control mice is inoculated from the same tumor suspension employed for the experiment. The groups in this set of controls receive a series of concentration levels of inoculum ranging above and below the inoculum level employed in the treatment groups. This set of controls is observed for survival time and percentage "takes," and indicates the potency of the inoculum level employed in the treatment groups. It permits a comparison between the results obtained employing a varying number of tumor cells in the inoculum with the antileukemic action of the drug treatment.

#### A. 6-Mercaptopurine plus Adenylic Acid

In two experiments, the antileukemic specificity of action of 6-mercaptopurine, expressed in terms of the relative effect of the drug on the tumor and host, was not altered by administration of adenylic acid. In the first experiment, the adenylic acid was administered concomitantly with 6-mercaptopurine (FIGURE 1). In the second experiment, the administration of adenylic acid was withheld for one hour (FIGURE 2).

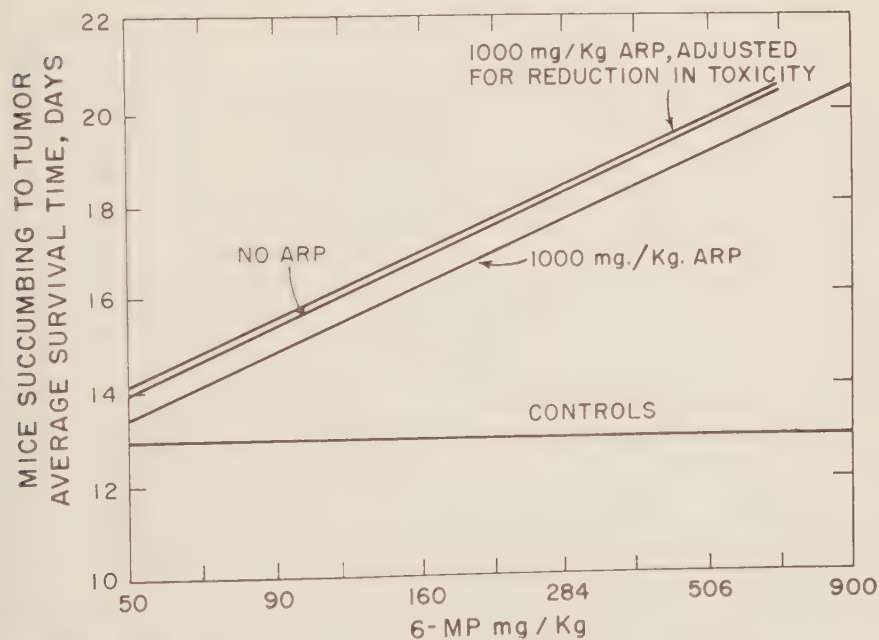


FIGURE 1. Effect of concomitant administration of adenylic acid (ARP) on the survival time—6-mercaptopurine (6-MP) dosage relationship in leukemic mice. Fitted least squares common slope lines are plotted. Adjustment for reduction in 6-MP toxicity due to adenylic acid is based on the relative increase in the 6 MP LD<sub>50</sub>. Tumor implant  $1.06 \times 10^6$  cells per mouse. Treatment two days following tumor implant. Adenylic acid S.C., 6-MP I.P. Two mice with high doses of 6-MP alone (506 mgm./kg.; 213 mgm./kg.) show no evidence of tumor 85 days following implant. They are not included in the calculation of survival time. In the tumor titration 100 per cent takes was observed with  $1.06 \times 10^6$  cells per mouse.



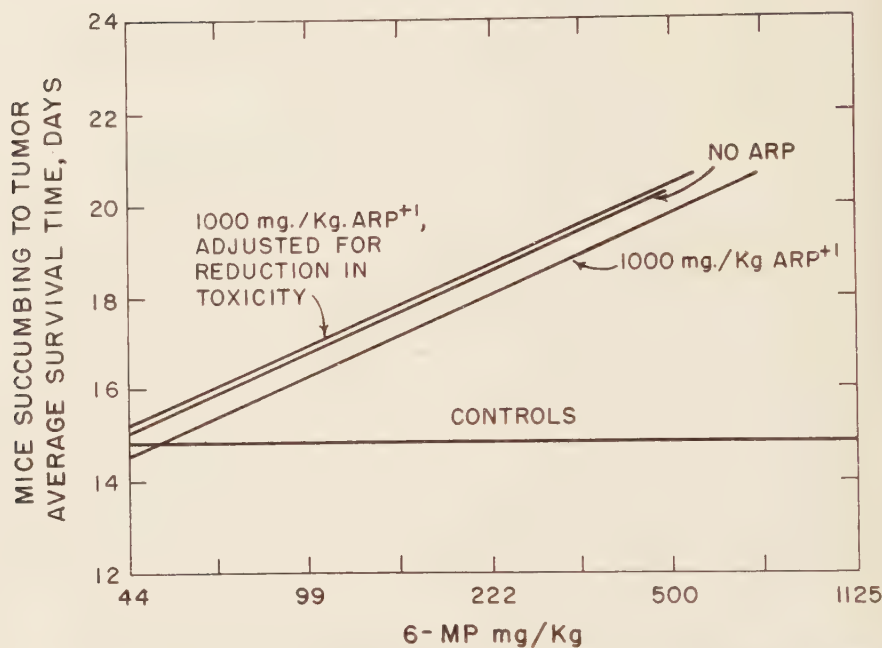


FIGURE 2. Effect of one hour delayed adenylic acid (ARP) on the survival time—6-mercaptopurine (6-MP) dosage relationship in leukemic mice. Fitted least squares common slope lines are plotted. Adjustment for reduction in 6-MP toxicity due to adenylic acid is based on the relative increase in the 6-MP  $LD_{50}$ . Tumor implant  $1.1 \times 10^6$  cells per mouse. Treatment 3 days following tumor implant. Adenylic acid S.C., 6-MP I.P. In the tumor titration 100% takes was observed with  $1.1 \times 10^6$  cells per mouse.

The dose-mortality relationships with 6-mercaptopurine administered alone and with metabolite appeared to be similar in mice with and without tumor. The increase in median lethal dose on administration of adenylic acid with the 6-mercaptopurine did not differ essentially in mice with and without tumor.

The survival time of mice that succumbed to leukemia increased with the dose of 6-mercaptopurine, when employed alone or with adenylic acid (FIGURES 1 and 2). Administration of adenylic acid concomitantly or one hour subsequent to 6-mercaptopurine afforded protection to the tumor as well as to the host for a specified dose of 6-mercaptopurine. The extent of protection for host and tumor was essentially proportional. The protection afforded by adenylic acid to the host accordingly permitted the employment of higher doses of 6-mercaptopurine. However, this gain was canceled by the reduction in the effectiveness of 6-mercaptopurine against the tumor. When a comparison is made at equal cost in drug-dose mortality response, no appreciable gain in survival time is noted (FIGURES 1 and 2).

The two experiments just reported were performed without the knowledge, subsequently gained, of the potentiation of adenylic acid toxicity by 6-mercaptopurine. Any capacity of adenylic acid to increase the antileukemic specificity of 6-mercaptopurine may have been obscured in these experiments by the heightened adenylic acid toxicity. These experiments bear repeating with lower doses of adenylic acid. The role of temporal relationships of administra-

tion of the drugs will be investigated further. The influence of other effective metabolites on the antileukemic specificity of action of 6-mercaptopurine has not as yet been investigated.

### B. Schedule of Treatment

Several experiments were conducted to determine whether the antileukemic specificity of action of 6-mercaptopurine may be altered by the schedule of treatment. When comparisons were made at equal cost in drug-dose mortality, several multiple treatment schedules were more effective than treatment on a single day in increasing the survival time of the leukemic mice: (a) two treatments spaced four days apart (days 2 + 6 following tumor inoculation) were more effective than treatment on day 2 alone or day 6 alone in increasing the survival time of the leukemic mice (FIGURES 3 and 4); (b) treatments spaced four days apart (days 3 + 7; days 3 + 7 + 11) and treatments spaced one day apart over corresponding total intervals (days 3 through 7; days 3 through 11) were essentially equally effective at equal cost in host mortality in increasing the survival time of the leukemic mice (FIGURES 5 and 6). All of these multiple treatments were more effective than a single treatment (day 3). See

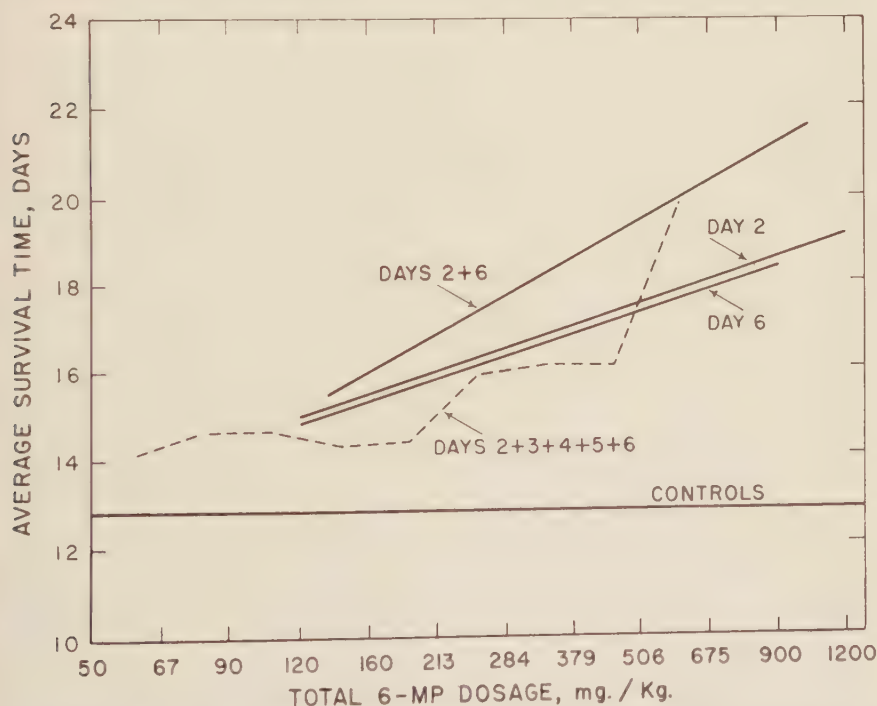


FIGURE 3 (*cf.* FIGURE 4). Relation of average survival time, for mice succumbing to leukemia, to the total 6-mercaptopurine (6-MP) dosage. Fitted least squares lines are plotted for treatment on the following days after tumor implant: day 2; day 6; days 2 + 6. For the treatment group, days 2-6, a least squares fit was not employed since many of the doses were below the linear range. Tumor implant  $1.18 \times 10^6$  cells per mouse. 6-MP I.P. Two mice, treated on days 2 + 6 with a total of 379 mgm./kg., 6-MP, show no evidence of tumor 71 days following implant. They are not included in the calculation of survival time. In the tumor titration 100 per cent takes was observed with  $1.18 \times 10^6$  cells per mouse.

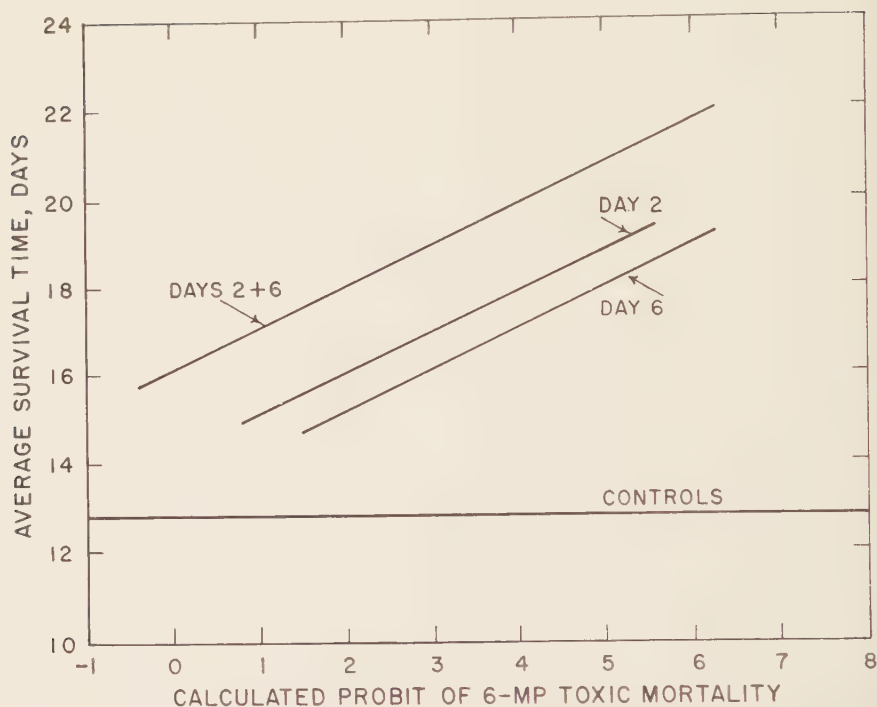


FIGURE 4 (*cf.* FIGURE 3). Effect of schedule of treatment on the survival time—toxic mortality relationship in leukemic mice. Fitted least squares common slope lines are plotted. Data for the treatment group, days 2-6, cannot be represented since no toxic mortality was observed at any dose employed. It was not anticipated that a divided dose schedule would result in the observed sharp increase in the tolerance for 6-MP.

FIGURES 5 and 6. With the multiple treatment schedules employed, in general, the total dose-mortality response (probit) curves and the total dose-survival time curves had steeper slopes than those observed for single treatment. The survival time-probit curves remained essentially parallel since the increases in slope with respect to host and tumor response on multiple treatment were essentially proportional.

It is of interest to note that with either aminopterin or A-Methopterin, daily multiple treatment was not as effective as multiple treatment spaced four days apart.<sup>20</sup> With these drugs there was a sharp rise in toxicity on daily treatment.<sup>11, 20</sup> In contrast with the "antifolics," the schedule of multiple treatment necessary for enhanced antileukemic effect with 6-mercaptopurine appears to be less critical. With 6-mercaptopurine, the total dose LD<sub>50</sub> for daily treatment was actually somewhat higher than that for single treatment or for treatment every four days over the same total interval (FIGURE 5).

The total dose of 6-mercaptopurine may be increased not only by increasing the dose level for a single treatment but also by increasing the number of treatments at a given dose level. In each situation, the extent of increase in survival time is limited by the toxicity to the host. Although multiple daily treatments and treatments spaced four days apart over the same total interval

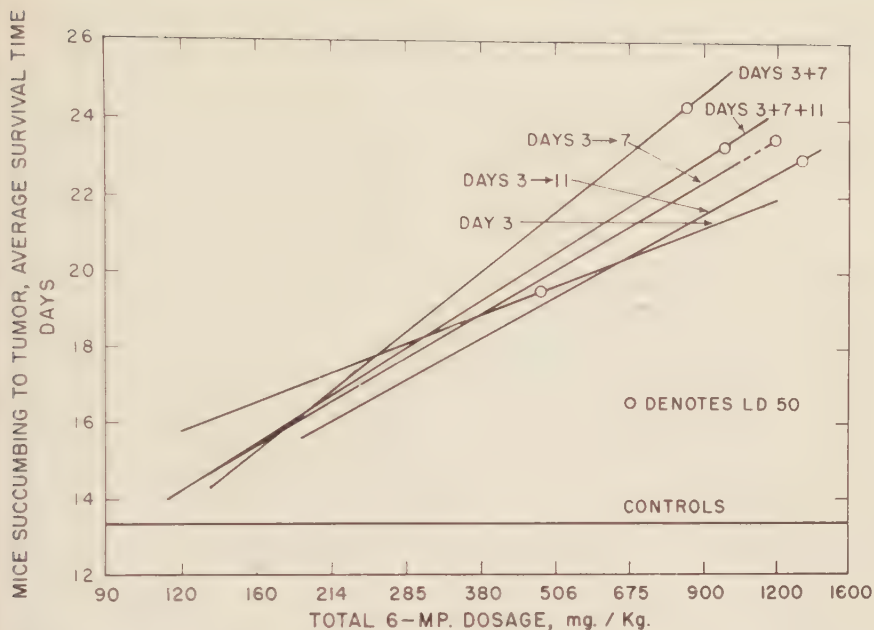


FIGURE 5 (cf. FIGURE 6). Relation of average survival time, for mice succumbing to leukemia, to the total 6-mercaptopyrine (6-MP) dosage. Fitted least squares lines are plotted for each of the treatments employed. The treatment days indicated are the days following tumor implant. Tumor implant  $1.11 \times 10^6$  cells per mouse. 6-MP I.P. In the tumor titration 100% takes was observed with  $1.11 \times 10^4$  cells per mouse.

Certain mice, with extensive survival times, have not been included in the calculations made. These include: days 3 + 7, 380 mgm./kg., 1 mouse alive and tumor free on day 55; days 3 + 7 + 11, 285 mgm./kg., 1 mouse died on day 49; days 3-7, 214 mgm./kg., 2 mice alive and tumor free on day 55—160 mgm./kg., 1 mouse died on day 37; days 3-11, 160 mgm./kg., 1 mouse died on day 37 and another on day 49.

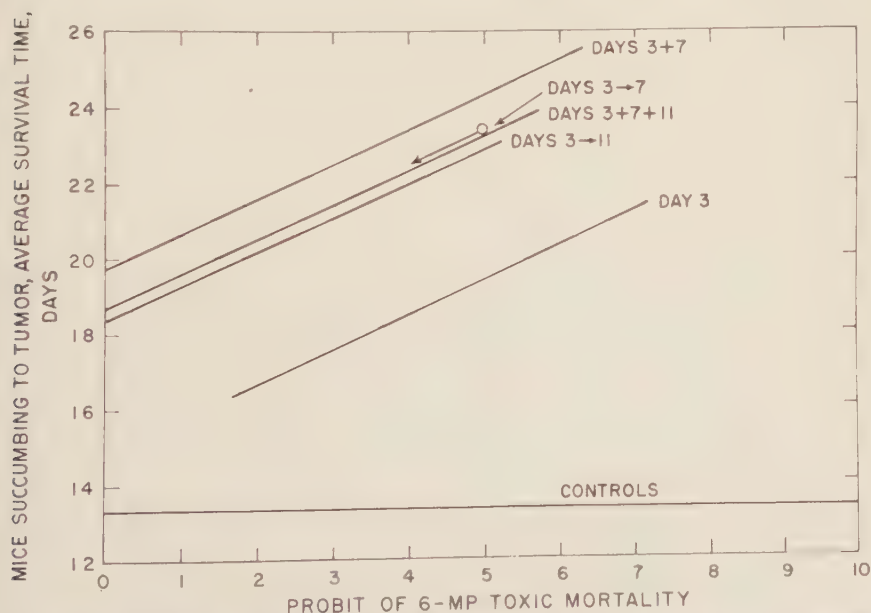


FIGURE 6 (cf. FIGURE 5). Effect of schedule of treatment on the survival time—toxic mortality relationship in leukemic mice. Fitted least squares common slope lines have been modified to force the lines through the LD<sub>50</sub> points represented in FIGURE 5.



TABLE 5  
RESULTS OF EXTENDED TREATMENT OF LEUKEMIC MICE WITH 6-MERCAPTOPURINE

Interval between treatments (days)	Size of treatment mgm./kg.	Duration of treatment (days)	Percent toxic deaths	Day of death of individual mice, all causes
1	120	3-7	0	17, 19, 20, 20, 20, 20, 21, 21, 22, 31
1	120	3-11	20	15, 18, 20, 21, 22, 23, 24, 25, 25, 26
1	120	3-21	80	15, 19, 20, 20, 21, 21, 21, 22, 22, 22
1	90	3-7	0	15, 15, 17, 18, 18, 18, 18, 18, 18, 20
1	90	3-11	20	16, 17, 18, 20, 20, 20, 20, 21, 25, 32
1	90	3-23	10	17, 22, 22, 24, 24, 24, 25, 25, 26, 26
4	284	3-7	0	19, 21, 21, 21, 22, 22, 22, 22, 23, 24
4	284	3-11	30	14, 16, 16, 23, 24, 26, 26, 26, 28, 49
4	284	3-27	80	5, 5, 31, 31, 32, 32, 32, 34, 35, 38

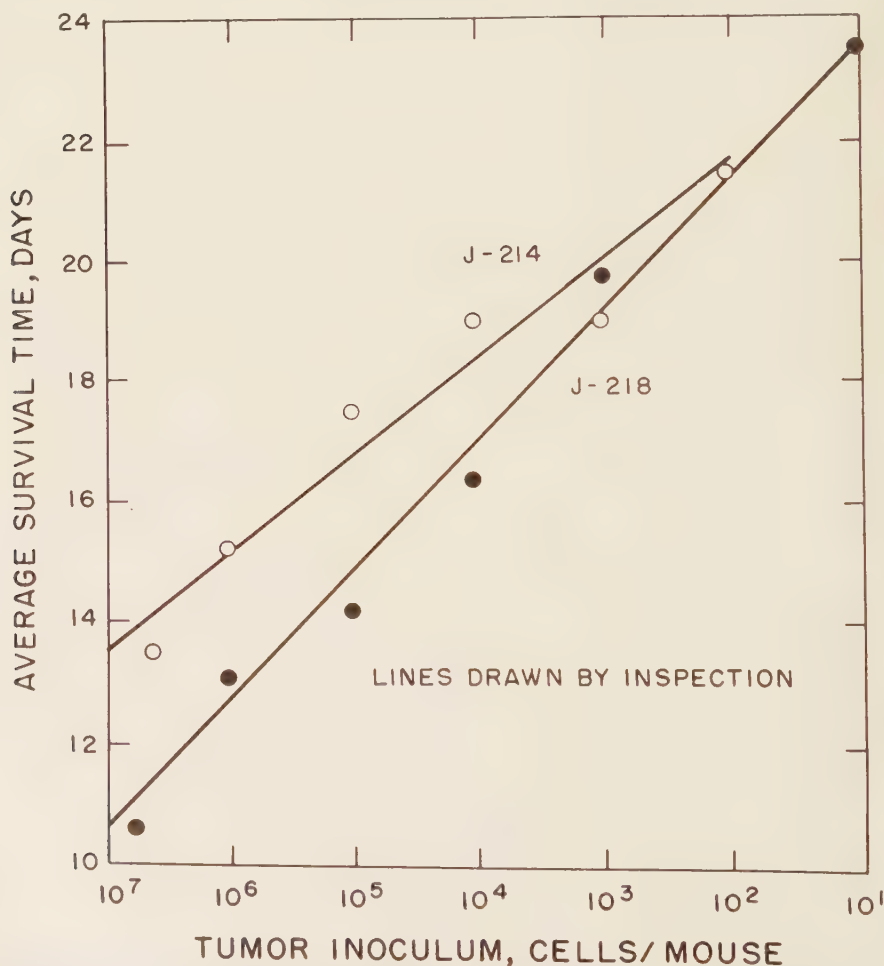


FIGURE 7 (cf. FIGURE 8). Relation between size of leukemia inoculum and survival time for mice succumbing to leukemia. Tumor titration in untreated mice. (J-218, cf. also FIGURES 1 and 9; J-214, cf. also FIGURES 2 and 10).

gave essentially equal increases in survival time at equal probit of toxicity, it is suggested that, with the longer interval between treatments (every four days), the drug treatment may be extended for a longer period of time, thus permitting more extensive increase in survival time. In a preliminary experiment investigating the effects of extended treatment (TABLE 5), with a dose of 284 mgm./kg. of 6-mercaptopurine administered every fourth day, a pronounced increase in survival time was accordingly evident when treatment was extended through the 27th day following tumor implant. With a dose of 120 mgm./kg. of 6-mercaptopurine administered daily, this increase was not so great when treatment was extended through the 21st day. A dose of 90 mgm./kg. of 6-mercaptopurine administered daily through the 23rd day was also less effective than multiple treatments spaced four days apart. Except for two mice dying early, the mice receiving 284 mgm./kg. of 6-mercaptopurine every fourth day from day 3 through 27 showed no evidence of local tumor and were in apparent good health up through about day 25. Subsequently, the mice showed toxic symptoms and all died by day 39, only 2 showing any evidence of local tumor. The group treated with 120 mgm./kg. of drug daily died with weight loss and little or no evidence of local tumor. The group

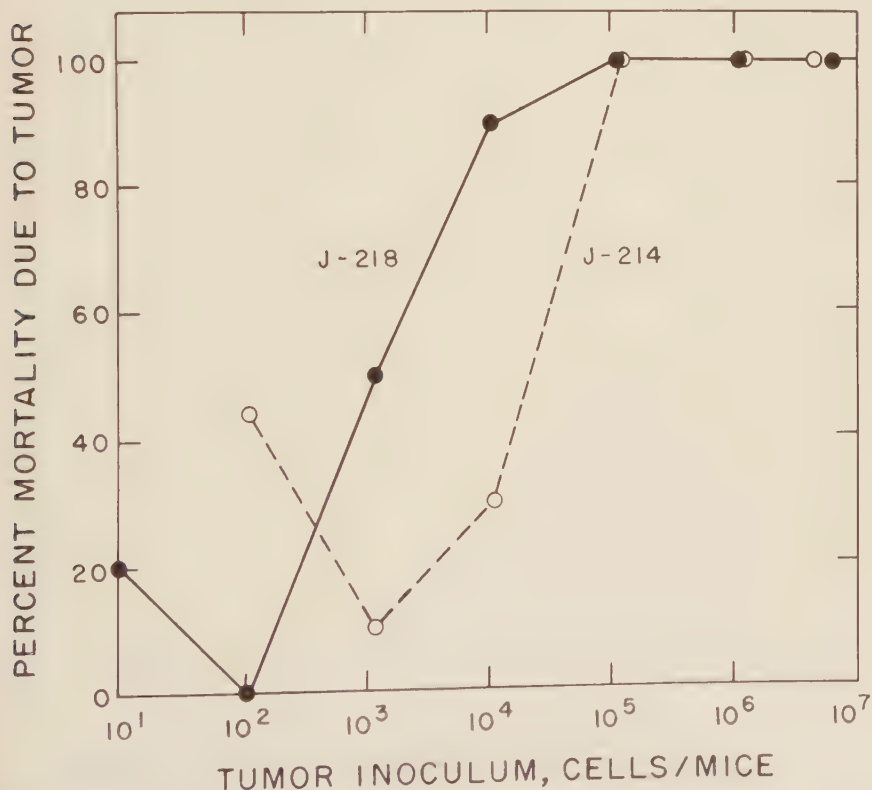
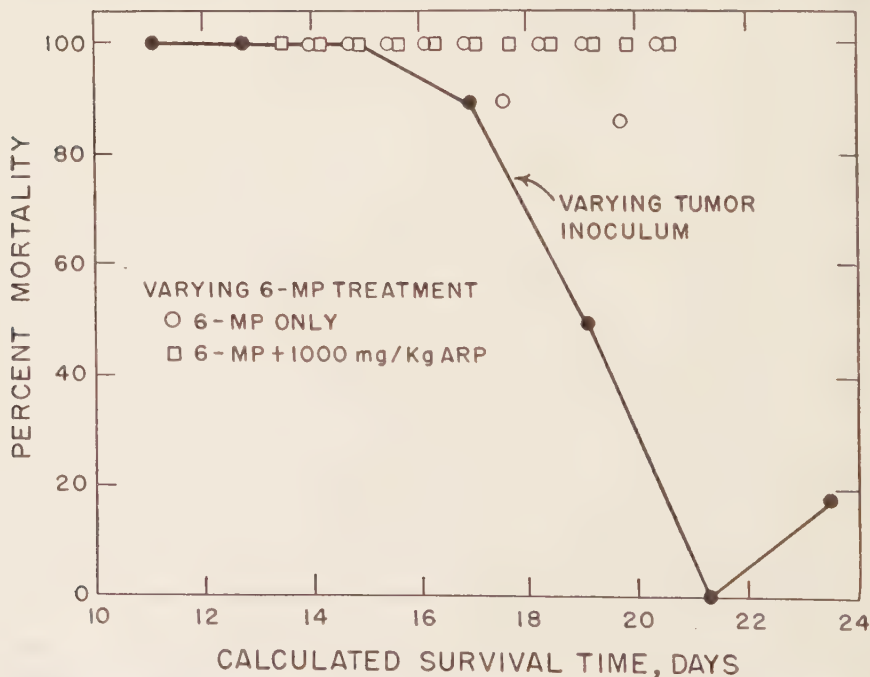


FIGURE 8 (cf. FIGURE 7). Relation between size of leukemia inoculum and proportion of mice succumbing to leukemia. Tumor titration in untreated mice. (J-218, cf. also FIGURES 1 and 9; J-214 cf. also FIGURES 2 and 10).

treated with 90 mgm./kg. of 6-mercaptopurine died with large local tumor and little or no evidence of weight loss. The data suggest that the effects of multiple-treatment schedules in influencing the antileukemic specificity of action of 6-mercaptopurine are worthy of further study.

### C. Survival Time and Tumor Mortality in Treated Animals and Controls

A comparison of the antileukemic action of 6-mercaptopurine with the results obtained with untreated controls in which varying numbers of tumor cells were employed in the inoculum is of interest. As the tumor inoculum concentration is decreased, the survival time is increased (FIGURE 7) and the percentage "tumor takes" is decreased (FIGURE 8). If the action of 6-mercaptopurine were to kill 6-mercaptopurine-sensitive tumor cells, leaving other tumor cells unaffected, one would expect its use to give a similar result, as though a smaller tumor inoculum were employed, but without 6-mercaptopurine treatment. In FIGURES 9 and 10, both the varying tumor inoculum (tumor titration) and the fixed tumor inoculum with varying 6-mercaptopurine are expressed in terms of the calculated survival time to which they give rise. Against these survival times are plotted the percentage of animals (excluding drug toxicity deaths) dying of tumor. It is evident that with varying 6-mercaptopurine there is a greater proportion of animals dying from tumor for a given survival time than from varying inoculum. This result suggests that the



FIGURES 9 (cf. FIGURES 1, 7, 8). Relation between proportion of mice succumbing to leukemia and survival time of such mice. The relation when survival time is extended by antileukemic treatment is contrasted with that applying when survival time is extended owing to reduced leukemia implant.

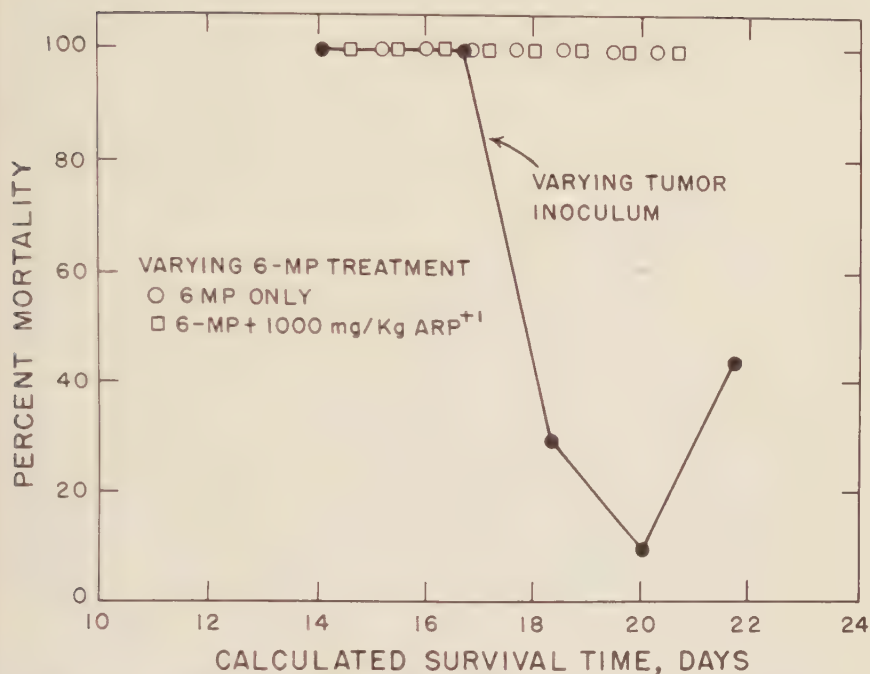


FIGURE 10 (cf. FIGURES 2, 7, 8). Relation between proportion of mice succumbing to leukemia and survival time of such mice. The relation when survival time is extended by antileukemic treatment is contrasted with that applying when survival time is extended owing to reduced leukemia implant.

effect of 6-mercaptopurine is to cause graded damage to the tumor cells, rather than simply to destroy or leave unaffected each individual tumor cell.

It has been observed in this laboratory that, with Aminopterin and A-Methopterin, as well as with 6-mercaptopurine, it is possible to increase the antileukemic specificity of action of the drugs by the employment of appropriate schedules of treatment. With Aminopterin, the antileukemic specificity was also increased by the delayed administration of citrovorum factor. With the above treatments, there has been a low but persistent percentage of "no takes" with leukemia 1210 in experiments in which treatment was initiated early. This result has occurred in carefully controlled experiments in which the inoculum level employed in the experiment was well above the level of tumor inoculum giving 100 per cent takes in untreated animals. It is indicated that these "no takes" actually represent total recovery from the leukemic implant. Further investigation of the factors influencing the antileukemic specificity of action of drug treatment could conceivably lead to the development of more effective treatment procedures, with increasing percentages of "no takes" or recoveries, and with little or no cost in toxic mortality.

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# EFFECTS OF 6-MERCAPTOPURINE ON EXPERIMENTAL TUMORS

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Elion and Hitchings<sup>1</sup> first prepared 6-mercaptopurine and found it to be a purine antagonist in *Lactobacillus casei* reversible by adenine, guanine, xanthine, and hypoxanthine, but not by folic acid.<sup>2</sup> They also observed that resistance of *L. casei* to 6-mercaptopurine was not accompanied by resistance to adenine antagonists such as 2,6-diaminopurine, 8-azaadenine, and purine, or to the guanine antagonist 8-azaguanine<sup>3</sup>.

Clarke and his co-workers<sup>4</sup> have reported on the effects of 6-mercaptopurine against sarcoma 180 which were characterized by only moderate inhibition during the initial period of treatment but, more significantly, by the eventual nonviability of the tumor as measured by transplantation and a significant number of complete tumor regressions.

Burchenal *et al.*<sup>5</sup> have observed that 6-mercaptopurine will cause temporary remissions in certain cases of acute human leukemia, including those which have become resistant to A-Methopterin.

The present paper presents certain additional results which have been obtained with 6-mercaptopurine against several mouse neoplasms, including leukemias which are resistant to certain anticancer agents.

## *Experimental*

*Solid tumor studies.* Using the appropriate strains of mice, we have assayed the inhibitory effects of 6-mercaptopurine against the solid tumors adenocarcinomas 755 and Eo 771 and sarcoma 180. Typical results are summarized in TABLE 1.

It may be seen from data presented in TABLE 1 that when treatment was begun, 24 hours after tumor implantation, 6-mercaptopurine almost completely inhibited growth of adenocarcinoma 755 for 12 days. The weights of the 755 tumor in treated mice were approximately the same as the pieces trocarred 12 days earlier. This profound inhibition may be attained with doses of one fifth the maximum tolerated dose. 6-Mercaptopurine, in our hands, was only moderately effective in preventing growth of adenocarcinoma Eo 771 and sarcoma 180.

Employing adenocarcinoma 755, experiments were then carried out to determine the effects of 6-mercaptopurine when treatment was delayed. The results of these experiments are presented in TABLE 2.

The results presented in TABLE 2 indicate that 6-mercaptopurine causes immediate cessation of rapidly growing adenocarcinoma 755 if treatment is delayed as long as eight days, but no rapid "melting away" of the neoplasm was observed.

Following the lead of Clarke *et al.*<sup>4</sup> experiments were carried out on the

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TABLE 1  
EFFECTS OF 6-MERCAPTOPYRINE ON SEVERAL SOLID TUMORS IN MICE

Tumor	Treatment	Dosage (mgm./kg.)	Avg. tumor wt. (mgm.)	% of controls	Avg. animal wt. change (gm.)
755	Controls	—	764	—	+2.4
	6-MP	40 (×6)	12	2	-0.4
	6-MP	20 (×6)	24	3	-0.2
	6-MP	10 (×6)	23	3	0
Eo 771	Controls	—	1032	—	+3.9
	6-MP	40 (×6)	486	47	+0.8
	6-MP	20 (×6)	354	34	+1.0
	6-MP	10 (×6)	549	53	+2.1
Sa 180	Controls	—	1298	—	+4.1
	6-MP	40 (×5)	413	32	+3.2
	6-MP	20 (×5)	327	25	+4.9
	6-MP	10 (×5)	597	46	+3.0

Note. Treatment was initiated at 24 hours after tumor implantation and continued on an alternate day basis for the total number of injections indicated in parenthesis. Experiments with sarcoma 180 were terminated on the 8th day, adenocarcinomas 755 and eo 771 on the 12th day. Ten mice were used in all experiments.

viability of tumor 755 following treatments with 6-mercaptopurine. In these experiments a comparison between 6-mercaptopurine and 8-azaguanine was made with regard to tumoricidal action on adenocarcinoma 755. Results obtained are presented in TABLE 3.

The results presented in TABLE 3 suggest that 6-mercaptopurine at the maximum tolerated dose provides for *complete inhibition* of tumor 755 and cytotoxic effects on the small tumor implant when treatment is initiated at 24 hours and continued every other day for a total of six injections. Under similar conditions 8-azaguanine was only carcinostatic and, when treatment was stopped, rapid tumor growth resumed.

TABLE 2  
EFFECTS OF 6-MERCAPTOPYRINE ON ADENOCARCINOMA 755 WHEN TREATMENT IS DELAYED

Exper. No.	Treatment	Dosage (mgm./kg.)	Day treatment initiated	Day tumors were weighed	Avg. tumor wt. (mgm.)	% of control
1	Control	—	—	12	716	—
	6-MP	30 (×4)	5	12	31	4
	6-MP	30 (×2)	9	12	466	65
2	Control	—	—	12	1527	—
	Control	—	—	8	148	—
	6-MP	40 (×4)	8 (a)	12	165	11
	6-MP	40 (×6)	1	12	14	1

Note. All animals were sacrificed and tumors weighed on the 12th day with the exception of the 8-day controls in Experiment No. 2 (included as a reference point for the experiment in which 6-mercaptopurine treatment was initiated on the 8th day). All treatment was on an alternate day basis, starting when indicated with the exception of (a) where treatment was daily (8th, 9th, 10th, and 11th days).

TABLE 3  
TUMORICIDAL ACTION OF 6-MERCAPTOPURINE AND 8-AZAGUANINE IN MICE BEARING  
ADENOCARCINOMA 755

Treatment	Dosage (mgm., kg.)	Days to reach final tumor wt.	Avg. final tumor wt. (mgm.)	No. of mice with no tumor	Mortality	Avg. animal wt. change (gm.)
Controls	—	12	1174	0/14	0/14	+4.7
6-MP	40	25	0	8/8	2/10	+2.8
	20	25	187	5/10	0/10	+4.1
	10	25	1039	5/10	0/10	+1.3
8-8-Azaguanine	75	17	945	0/10	0/10	+2.2
	37	17	1144	0/10	0/10	+3.5
	18	17	1332	0/10	0/10	+5.5
6-MP + 8-Aza- guanine	40 + 75	25	115	6/8	2/10	+1.2
	20 + 37	25	136	5/9	1/10	+1.9
	10 + 18	25	1276	2/10	0/10	+2.0

Note. Above animals were treated as indicated on 1, 3, 5, 7, 9, and 11 days following tumor implantation. All treatment was stopped on the 11th day and tumor size was followed by external measurement until they reached approximately that of the control at 12 days or until extirpation at 24 days.

#### EFFECTS OF 6-MERCAPTOPURINE ON CERTAIN MOUSE LEUKEMIAS

Utilizing several drug-resistant lines of L1210 leukemia generously supplied us by Doctor L. W. Law, we have carried on certain studies with 6-mercaptopurine. The results of these efforts are summarized in TABLE 4.

It is also of interest to note the effects of 8-azaguanine on these lines of L1210 leukemia, TABLE 5.

#### *Effects of 6-Mercaptopurine in Combination with Other Agents Against Mouse Leukemias*

Several groups have reported that the combination of A-Methopterin plus 8-azaguanine is more effective in increasing life span of mouse leukemia L1210 than either drug alone. In cooperation with Doctor Hitchings and Miss Elion, we have compared the effects of 6-mercaptopurine plus A-Methopterin against L1210 leukemia.<sup>6</sup> The results of these studies are shown in TABLE 6.

In our hands 6-mercaptopurine has failed to increase significantly the life span of AkR mice with L4946 leukemia.

The results presented in TABLE 6 and other results obtained in this laboratory suggest the possibility that 6-mercaptopurine plus A-Methopterin given in combination may be somewhat more effective against L1210 leukemia than either agent given alone at the maximum tolerated dose.

In experiments employing solid tumors sarcoma 180 and adenocarcinomas 755 and Eo 771 no indication of synergism between 6-mercaptopurine and A-Methopterin has been observed.

Stock *et al.*<sup>7</sup> have shown that azaserine potentiates the antitumor activity of 6-mercaptopurine against sarcoma 180. Following this observation, we have carried out experiments comparing the effects of 6-mercaptopurine and



TABLE 4

EFFECTS OF 6-MERCAPTOPURINE ON THE LIFE SPAN OF MICE WITH SEVERAL DRUG-RESISTANT LINES OF L1210 LEUKEMIA

Leukemia	Dosage (mgm./kg.)	Avg. life span (days)		% Above controls
		Controls	Treated	
L1210	40	10.6	16.0	+62
	30	11.6	17.1	+47
	20	10.6	12.6	+19
L1210-A-Methopterin-dependent	50	11.6	9.3	-20
	40	7.7	7.3	-5
	40	12.4	11.5	-1
	40	9.9	8.3	-16
L1210-8-Azaguanine-dependent	40	15.2	14.2	-9
	40	11.7	7.2	-38
	40	14.1	8.9	-63
6-MP-resistant	40	9.1	8.7	-4
	40	7.4	8.1	+9

Note. All treatment begun 24 hours after inoculation of leukemic cells and continued every other day for a total of 10 injections or until death.

TABLE 5

EFFECTS OF 8-AZAGUANINE ON THE LIFE SPAN OF MICE WITH SEVERAL DRUG-RESISTANT LINES OF L1210 LEUKEMIA

Leukemia	Dosage (mgm./kg.)	Avg. life span (days)		% Above controls
		Controls	Treated	
L1210	75	10.6	19.1	+80
	50	11.6	20.0	+72
	37	10.6	16.8	+58
L1210-A-Methopterin-dependent	75	8.7	13.4	+54
	50	8.7	13.3	+53
	50	9.9	14.3	+44
8-Azaguanine-dependent	75	10.9	6.1	-44
	75	11.7	12.6	+7
	75	14.1	12.2	-13
6-MP-resistant	75	8.8	8.9	+1
	75	9.1	8.5	-7

Note. Treatment initiated at 24 hours and continued every day for a total of 20 injections or until death.

8-azaguanine in combination with azaserine. Results obtained are summarized in TABLE 7.

It appears from the data presented in TABLE 7 that the azaserine + 6-mercaptopurine combination is synergistic with regards to antileukemic activity (L1210 leukemia) and that the azaserine plus 8-azaguanine combination is also synergistic.

The 6-mercaptopurine + azaserine combination failed to show any indica-

TABLE 6

EFFECTS OF 6-MERCAPTOPURINE PLUS A-METHOPTERIN AGAINST LEUKEMIA L1210

Exper. No.	Dosage (mgm./kg.)		Life span		% Above controls
	A-Meth.	6-MP	Days	Range (avg.)	
1	—	—	10.2	10-12	—
	3	—	19.6	14-27	+92
	2	—	16.0	9-22	+57
	—	50	13.8	11-16	+35
	—	40	16.0	13-21	+56
	3	40	25.7	19-32	+152
	2	50	26.3	21-33	+159
	—	—	10.6	10-12	—
2	3	—	23.3	18-27	+120
	1.5	—	18.9	17-22	+78
	—	40	14.4	12-18	+36
	—	20	12.6	11-16	+19
	3	40	21.0	9-35	+98
	1.5	20	27.2	23-39	+156

Note. In all experiments treatment was initiated at 24 hours and continued every other day for a total of 10 injections. All combination treatment "simultaneous."

TABLE 7

EFFECTS OF 6-MERCAPTOPURINE AND 8-AZAGUANINE IN COMBINATION WITH AZASERINE

Dosage (mgm./kg.)			Life span		% Above controls
Azaserine	6-MP	8-Aza.	Days (avg.)	Range	
—	—	—	6.6	6-8	—
10	—	—	10.5	9-14	59
5	—	—	8.3	7-12	26
—	40	—	10.6	9-14	61
—	20	—	9.9	7-12	50
—	—	75	8.6	7-10	30
—	—	37	7.2	7-18	9
10	40	—	20.4	18-22	209
5	20	—	21.4	19-25	224
2.5	10	—	15.1	9-18	128
10	—	75	19.5	8-23	195
5	—	37	13.0	9-17	97
2.5	—	18	10.9	8-14	65

Note. All treatment initiated 24 hours after inoculation and continued every other day for a total of 10 injections or until death. 8-Azaguanine injected subcutaneously; azaserine and 6-MP injected I.P.

tion of potentiation in experiments employing leukemia L1946 in AKR mice where 6-mercaptopurine alone is without significant activity.

### Summary

(1) 6-Mercaptopurine is profoundly inhibitory to adenocarcinoma 755 and moderately inhibitory toward adenocarcinoma Eo 771 and sarcoma 180. This agent completely inhibits adenocarcinoma 755, if treatment is begun at 24 hours after transplantation, even at levels of one fifth the maximum tolerated

close. If treatment is delayed cessation of tumor growth may be achieved, but the well established tumor does not rapidly "melt away."

(2) In experiments with adenocarcinoma 755, it was demonstrated that 6-mercaptopurine is tumoricidal, whereas 8-azaguanine is tumoristatic under the selected conditions of test.

(3) 6-Mercaptopurine increases life span of mice with L1210 leukemia, but failed in our hands to affect the life span of mice with A-Methopterin-dependent and 8-azaguanine-dependent lines of L1210 leukemia. This purine antagonist fails to affect the life span of mice with leukemia L4946.

(4) Combinations of 6-mercaptopurine plus A-Methopterin or 6-mercaptopurine plus azaserine appear to be synergistic with regards to antileukemic activity (L1210 leukemia in mice).

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# THE EFFECTS OF 6-MERCAPTOPURINE, 8-AZAGUANINE, AND 1,4-DIMETHANESULFONYLOXYBUTANE ON AN EXPERIMENTAL BRAIN TUMOR: PRELIMINARY OBSERVATIONS\*

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In the study to be reported, the effects of 6-mercaptopurine (Purinethol, mercaptopurine), 8-azaguanine (guanazolo) and 1,4-dimethanesulfonyloxybutane (Myleran, GT41) on a transplantable brain tumor in mice were determined both *in vivo* and *in vitro*. The results have demonstrated that two of the drugs inhibit the growth of this malignant tumor. Evidence will be presented which suggests that a metabolic product of mercaptopurine may be the active antitumor agent, whereas the parent compound is inactive against this particular experimental neoplasm.

## *Materials and Methods*

*In vivo experiments.* The tumor used in this study has been designated Glioma 26 by Doctor Harry Zimmerman† of the Pathology Department, Montefiore Hospital, New York, N. Y. This particular tumor was induced by Doctor Zimmerman in C57 black mice by the intracerebral implantation of methylcholanthrene crystals according to the techniques described by him in a number of publications.<sup>1-4</sup> The histopathology of the tumor was that of a glioblastoma multiforme. The tumor originated in 1948 and has been carried by serial subcutaneous transplantations in C57 black mice for 135 generations.

Glioma 26 has been transplanted in our laboratory to the axillary region by the usual technique. Its natural history is characterized by progressive growth of the tumor without ulceration until the host dies from inanition two to two and one half months after transplantation.

In the experiments here reported, drug therapy was initiated one day after transplantation. The compounds were all administered once daily intraperitoneally for periods of 13 to 16 days. Mercaptopurine and 8-azaguanine were dissolved in an alkaline solution, as previously described,<sup>6</sup> with a final pH of 8. Myleran was dissolved in 30 per cent propylene glycol in water. Control tumor-bearing animals received intraperitoneal injections of the appropriate vehicle. At the termination of the experiment, the animals were killed with chloroform. The tumors were dissected out of the subcutaneous tissues and weighed promptly.

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† We wish to express our appreciation to Doctor Zimmerman for providing us with the tumor used in these studies.



TABLE 1  
EFFECT OF 6-MERCAPTUPURINE ON GLIOMA 26 IN C57 MICE

Exp. No.	Group	No. of animals	Dose of 6-MP mg./kg./day	Duration of therapy (days)	Mean tumor weight (mg.)	Standard deviation of mean (mgm.)	Avg. body weight (gm.)	
							Beginning	End
324	Control	10	—	—	574	136	16.0	17.6
	Treated	10	50	13	68	10	16.3	15.3
334	Control	9	—	—	532	116	16.4	19.7
	Treated	10	50	13	28	9	14.7	14.3
339	Control	10	—	—	600	87	15.7	17.0
	Treated	9	50	13	21	6	16.0	14.0
343	Control	10	—	—	471	90	16.6	19.4
	Treated	9	50	12	19	1	17.6	17.1
346	Control	10	—	—	693	133	16.4	18.2
	Treated	10	50	13	26	2	17.1	15.8

TABLE 2  
EFFECT OF 8-AZAGUANINE ON GLIOMA 26 IN C57 MICE

Exp. No.	Group	No. of animals	Dose of 8-aza. mgm./kg./day	Duration of therapy (days)	Mean tumor weight (mgm.)	Standard deviation of mean (mgm.)	Avg. body weight (gm.)	
							Beginning	End
298	Control	10	—	—	617	126	18.9	19.6
	Treated	10	50	16	1,182	215	16.7	17.8
324	Control	10	—	—	574	136	16.0	17.6
	Treated	9	50	13	444	72	16.2	15.9

TABLE 3  
EFFECT OF MYLERAN ON GLIOMA 26 IN C57 MICE

Exp. No.	Group	No. of animals	Dose of G.T. 41 mgm./kg./day	Duration of therapy (days)	Mean tumor weight (mgm.)	Standard deviation of mean (mgm.)	Avg. body weight (gm.)	
							Beginning	End
324	Control	10	—	—	574	136	16.0	17.6
	Treated	10	15	13	216	46	16.2	14.6
334	Control	9	—	—	532	116	16.4	19.7
	Treated	9	15	13	206	70	15.7	16.9

*In vitro experiments.* Mouse Glioma 26 was cultured in roller tubes for one to four weeks before transfer to slides for the evaluation of chemotherapeutic agents in order to obtain a more uniform selection of explants. The fibrinolytic activity of this tissue is so great that the routine plasma clot was lysed within 24 hours. Only through the addition of soybean trypsin inhibitor\* could a substantial outgrowth be maintained over the 9-day period. This required a minimal level of 0.025 mgm./cc. trypsin inhibitor at explantation and a carrying level of 0.15 mgm./cc. thereafter. At the same time, the plasma concentration was raised from 16 per cent to 50 per cent for normal

\* General Biochemicals, crystalline.

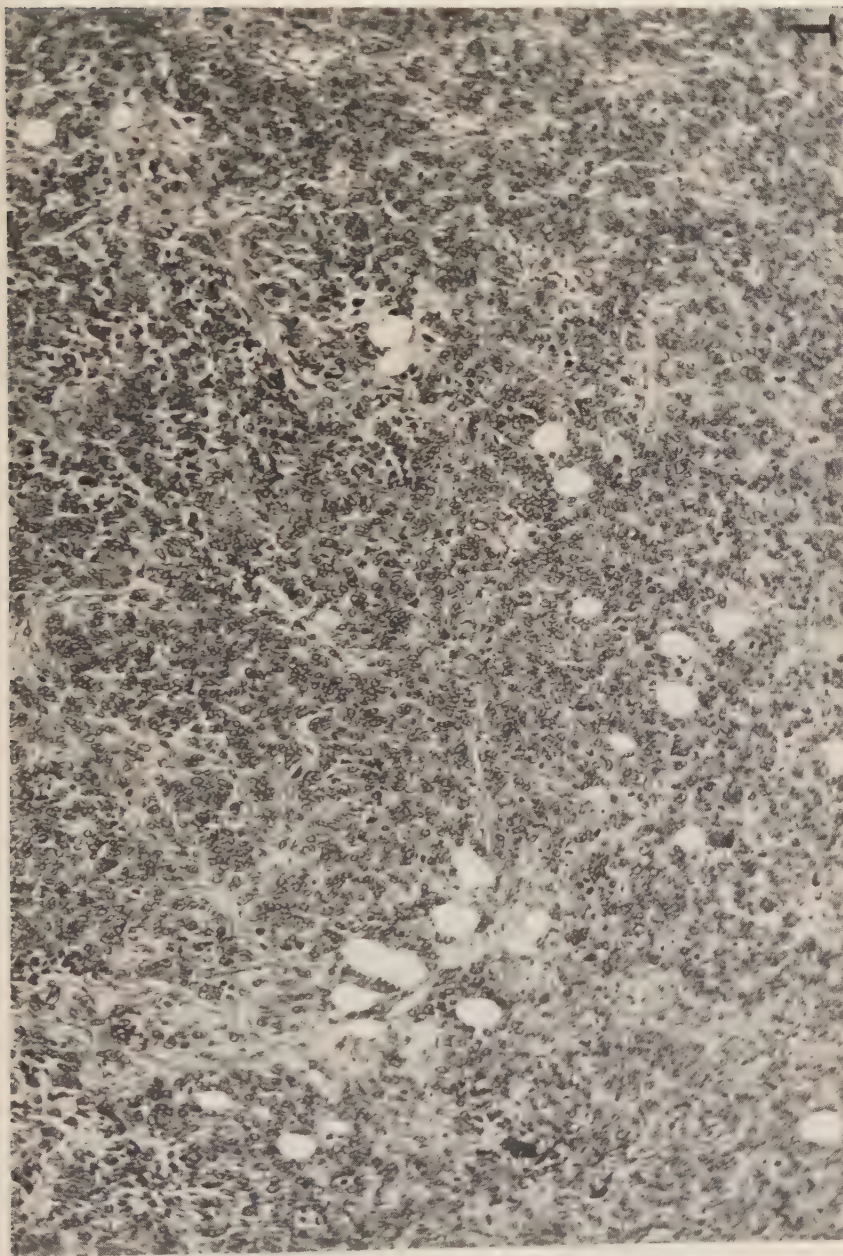


FIGURE 1. Glioma 26 transplanted to the subcutaneous tissue of a C57 black mouse. This section was taken from the same tumor which was then grown *in vitro* as shown in succeeding figures. Note the apparent uniformity of the cells here.  $\times 165$ .

TABLE 4  
EFFECT OF AGENTS ON GLIOMA 26 IN TISSUE CULTURE

Test agent	Concentration	No. of explants	Overall effect	Remarks
8-Azaguanine	M/100	12	0 to $\pm$	Some recovery at 7-9 days
"	M/200	12	0	
6-Mercaptopurine	M/100	28	0	
"	M/200	28	0	
Myleran	M/100	8	+	No recovery
"	M/200	8	+	No recovery
Propylene glycol*	3%	8	0	
"	1.5%	6	0	
Serum of 6-mercaptopurine-treated mice	—	20	0	
Serum of 6-mercaptopurine-treated mice deproteinized	—	16	0	
Control serum	—	20	0	
Control serum deproteinized	—	16	0	
Control	—	24	0	

\* Propylene glycol shows mild toxicity at seven days, whereas Myleran dissolved in this solvent is markedly inhibitory at five days. Although the possibility of synergism between the solvent and the drug cannot be eliminated on theoretical grounds, it is considered unlikely that the toxicity of the solvent contributes appreciably to the marked effect of the drug.

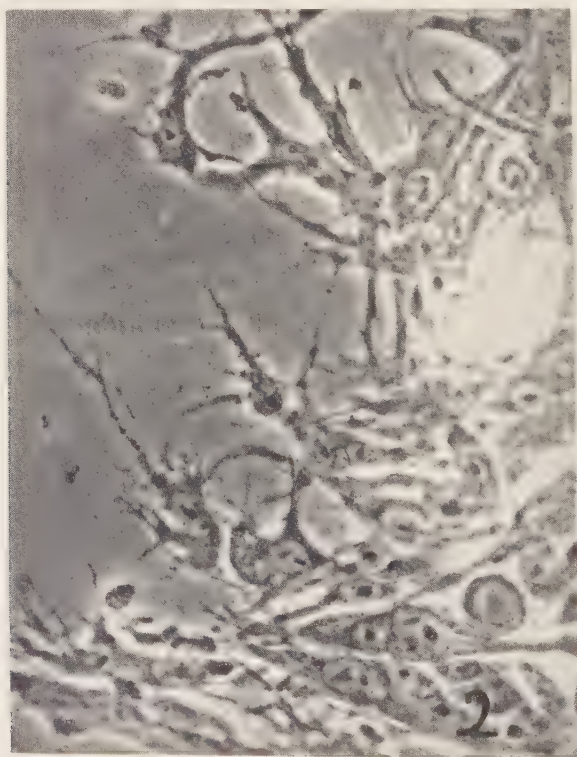


FIGURE 2. An area of glioma 26 in tissue culture. Note several typical astroblastic tumor cells at the periphery of the outgrowth. Phase contrast.  $\times 400$



clotting. Even in the presence of the trypsin inhibitor, one renewal of plasma was necessary. After 24 to 48 hours growth, the test compounds were incorporated into the feeding solution and renewed three times a week for seven to nine days. The culture medium for explantation consisted of one-half chicken plasma, one-third human placental serum, one-sixth chick-embryo extract, and trypsin inhibitor 0.025 mgm. cc.; the feeding solution was composed of three-fifths human placental serum, one-fifth ox ultrafiltrate, one-fifth chick-embryo extract, and trypsin inhibitor 0.15 mgm. cc. Stock solutions of 8-azaguanine and 6-mercaptopurine were prepared at  $\frac{M}{10}$  concentrations in sufficient NaOH to permit solution on heating. Both these compounds were tested at  $\frac{M}{100}$  and  $\frac{M}{200}$  levels by dilution in the feeding solution. 6-Mercaptopurine was further studied at  $\frac{M}{400}$ . Controls received NaOH equivalent to the  $\frac{M}{100}$  concentrations. Myleran was prepared at  $\frac{M}{10}$  concentration in 30 per cent propylene glycol and also tested at  $\frac{M}{100}$  and  $\frac{M}{200}$  levels. Controls of



FIGURE 3. Control culture of glioma 26 showing the large, often multinucleated cells which are commonly seen in this tumor. Phase contrast.  $\times 400$ .



propylene glycol comparable to both levels were included in the test runs since the toxicity of this solvent was unknown.

It was desired to study the *in vitro* antitumor activity of serum from mice which had been receiving mercaptopurine. In order to do so, mice were given injections of 50 mgm. per kilogram of 6-mercaptopurine twice daily for three days. They received a single dose on the fourth day and then, after ether anesthetization, were phlebotomized from the inferior vena cava under antiseptic precautions. The serum was used whole in one experiment. In another, the protein was removed by heat coagulation and the supernate was concentrated to half volume by vacuum distillation.

### Results

The results of the *in vivo* chemotherapeutic studies are summarized in TABLES 1, 2, and 3. In TABLE 1 it can be seen that Purinethol very effectively inhibited the growth of Glioma 26 at the dosage of 50 mgm. per kilogram. Although there was evidence of weight loss up to 12 per cent in one experiment, this was not consistent. In other observations not here reported, comparable and even greater weight loss failed to affect tumor growth.

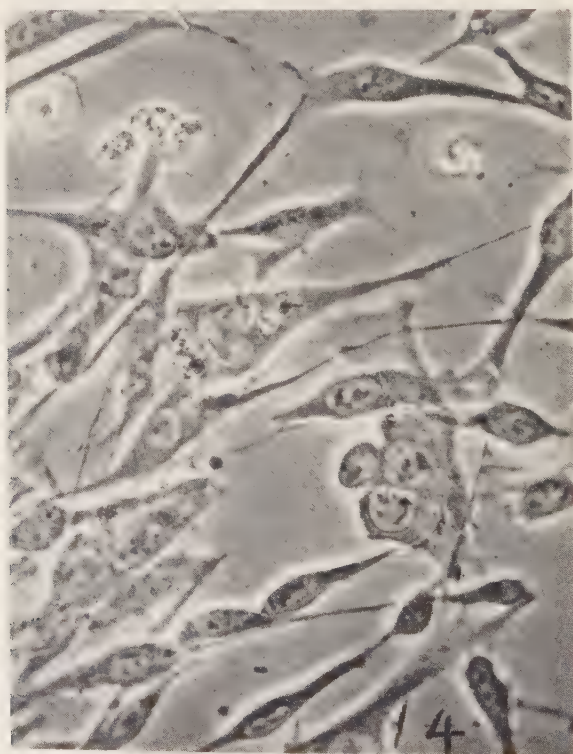


FIGURE 4. Control culture of glioma 26 showing spindle cells of varying size. Phase contrast.  $\times 400$ .

TABLE 2 summarizes two experiments in which 8-azaguanine failed, at essentially equimolar doses to mercaptopurine, to modify the growth rate of Glioma 26.

In TABLE 3, it is to be noted that Myleran, at daily doses of 15 mgm. per kilogram, inhibited the growth of the tumor significantly, though not as effectively as did mercaptopurine.

FIGURE 1 shows a photomicrograph of the tumor, which is a highly cellular, vascular neoplasm with apparent uniformity of the cell type. The actual variability in cellular type and the glial origin of these cells can be seen in the photomicrographs of this tumor in tissue culture. As shown by Russell and Bland<sup>5</sup> some morphological characteristics of this tumor type are often accentuated in tissue cultures.

There was no apparent change in the morphology of the tumor cells following mercaptopurine therapy *in vivo*. This paradox of marked tumor inhibition without significant histological change has been noted in other instances as well.<sup>6</sup>

The results of the *in vitro* experiments are summarized in TABLE 4 and FIG-

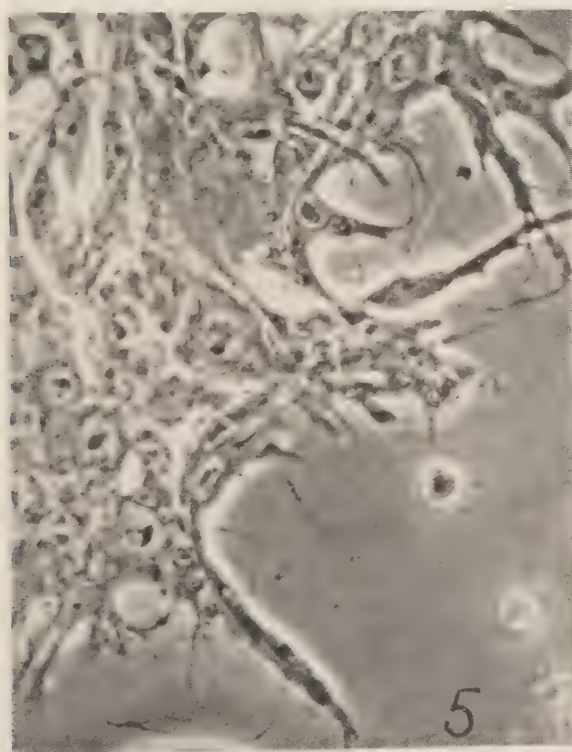


FIGURE 5. Glioma 26 after seven days exposure *in vitro* to 6-mercaptopurine at  $\frac{M}{200}$ , showing the absence of any deleterious effect. Phase contrast.  $\times 400$ .

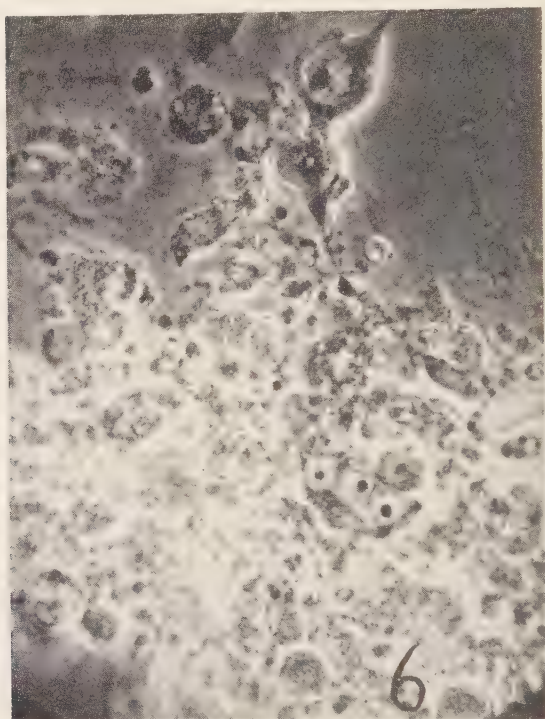


FIGURE 6. Glioma 26 after seven days exposure *in vitro* to Myleran at  $\frac{M}{200}$ , showing severe damage. Phase contrast.  $\times 400$ .

URES 2 to 7. The tumor cells of Glioma 26 grow either as discrete, broad, spindle-shaped cells, often with long thick processes, or as cohesive sheets or masses with more delicate branching terminal processes comparable to astroblasts. Multinucleated giant cells were also present in the culture. The photomicrographs and TABLE 4 present the evidence which leads to the conclusion that, *in vitro*, Purinethol and 8-azaguanine fail to affect significantly the cells of Glioma 26, whereas Myleran has a clearcut antitumor effect. It is to be noted that the drug concentration selected for this evaluation was  $\frac{M}{200}$  for each.

Since the *in vitro* effects of mercaptopurine are in such sharp contrast to its action *in vivo*, an attempt to demonstrate a therapeutically active metabolite in the serum was made. These experiments, summarized in TABLE 4, were negative. FIGURE 7 demonstrates a considerable increase in the deposition of lipid granules in the cytoplasm in cells exposed to the mouse serum. This may be reasonably explained as a manifestation of a less favorable medium than the controls, since the mouse serum replaced the human placental serum in these experiments. Probably no other significance should be attached to this nonspecific cellular change.

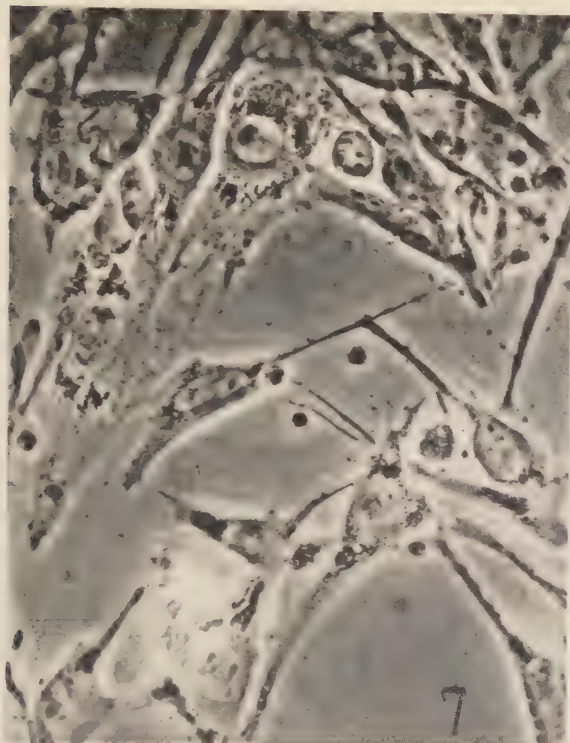


FIGURE 7. Glioma 26 cultured in mouse serum, showing considerable increase in the deposition of lipid granules. Phase contrast.  $\times 400$ .

### Discussion

The observations summarized in this report have demonstrated that 6-mercaptopurine and Myleran are effective tumor-inhibiting compounds against Glioma 26 *in vivo*, whereas 8-azaguanine is ineffective. It has been shown further that Purinethol fails to affect this tumor *in vitro*, whilst Myleran has an obvious deleterious effect, and 8-azaguanine is ineffective both *in vivo* and *in vitro*. The discrepancy between the *in vivo* and *in vitro* actions of mercaptopurine suggests that the antitumor action may be attributable to the metabolism of the parent compound to a pharmacologically active metabolite. If it is assumed that mercaptopurine, following injection, is uniformly distributed in the body water, it follows that the tumor cells *in vivo* are exposed to approximately one tenth as much mercaptopurine as the tumor cells *in vitro* at the dosage levels which were employed. In view of the rapid degradation of the drug *in vivo*,<sup>7</sup> the effective concentrations at the cellular level are probably even lower.

An attempt was made to produce direct evidence for the presence of a circulating, therapeutically active metabolite by exposing Glioma 26 cells *in vitro* to the serum of mice previously given mercaptopurine. Had the experiment



been positive, the results would have been significant. Negative results do not have comparable value, since many explanations for the failure of the test are apparent.

The results, *in vivo* and *in vitro*, of 1,4-dimethanesulfonyloxybutane on Glioma 26 are of interest in themselves. In addition, the good correlation between the effects of Myleran and 8-azaguanine in the animal and in tissue culture further highlight the discrepancy of the mercaptopurine observations in the two systems.

### Summary

The growth of Glioma 26, a transplantable mouse brain tumor, is inhibited *in vivo* by 6-mercaptopurine and 1,4-dimethanesulfonyloxybutane, but not by 8-azaguanine. The growth of this tumor *in vitro* is also inhibited by 1,4-dimethanesulfonyloxybutane, but not by 6-mercaptopurine or 8-azaguanine. The difference in effectiveness of 6-mercaptopurine *in vivo* and *in vitro* is attributed tentatively to the formation of a pharmacologically active metabolite from the parent compound in the body. However, preliminary attempts to inhibit the growth of the tumor *in vitro* with serum from mice previously treated with the parent compound have been unsuccessful.

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# THE TOXIC EFFECTS OF 6-MERCAPTOPURINE AND RELATED COMPOUNDS\*

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The present review of the toxic effects of 6-mercaptopurine (6-MP) in experimental mammals has two primary aims. The first and more practical purpose concerns risks to be anticipated during therapeutic use. The second aim concerns the specific cytotoxic effects of 6-MP as a possible contribution to the elucidation of its mechanism of action. As such, the study is a continuation of work of this laboratory by which it has been possible to differentiate the cytotoxic effects of folic acid antagonists such as aminopterin<sup>1</sup> and certain diaminopyrimidines<sup>2-24</sup> from those of the nitrogen mustards and mustard-like compounds, such as HN2 and TEM,<sup>5</sup> and from those of 2,6-diaminopurine.<sup>4</sup> In the present instance, tissue changes induced by 6-MP are compared with those caused by 2,6-diaminopurine (2,6-DAP) and by other purine analogs. Certainly if the action of 6-MP is to be explained in terms of a specific metabolite antagonism, then such an exposition must draw sustenance not only from differences in toxic properties between 6-MP and, for example, 2,6-DAP, but must also reconcile similarities in the effects of these compounds.

*Toxicity studies.* FIGURE 1 provides a list of the purines employed and presents graphically their relative potency in terms of toxicity in Swiss male mice. In most instances, two schedules of dosage have been used, *i.e.*, single and five successive daily injections. The agents are listed in an order largely based on their relative potency by chronic administration. The bars topped by arrows refer to the fact that the highest dose employed, the quantity indicated by the height of the serrated column, is less than LD<sub>50</sub>. Data are taken from previous reports<sup>1-5, 6, 7</sup> and from unpublished observations of the authors.<sup>8</sup>

The graph illustrates that there is more than a hundredfold difference in potency between the two extreme members of the series. Moreover, 6-MP is neither the most nor least toxic of its close structural analogs. The extent by which the toxicity of each compound is enhanced by cumulative action can be appreciated by comparing the LD<sub>50</sub> of single doses with that of multiple doses. It may be seen that most of the compounds, including 6-MP, exhibit relatively minor cumulative activity, *i.e.*, the LD<sub>50</sub> in mgm./kg./day for five daily doses, when expressed as a fraction of the single median lethal dose, ranges between one fourth in the case of adenine to more than one half in the case of 6-chloro-

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The authors are greatly indebted to a number of investigators for samples of the various purines employed in the present study: to Doctor G. H. Hitchings, Miss G. B. Eliot, and their associates of The Wellcome Research Laboratories for 6-mercaptopurine, 2,6-diaminopurine, thioguanine, and 6-methylpurine; to Doctor A. Bendich, Doctor P. J. Russell, Jr., and Doctor J. J. Fox of The Sloan-Kettering Institute for 6-chloropurine and purine; and to Doctor G. B. Brown and Doctor J. Daxell of the Sloan-Kettering Institute for 2-chloroadenine and 2-chloro-8-oxadenine.

† With the assistance of Barbara Wheelock and Barbara Bond.

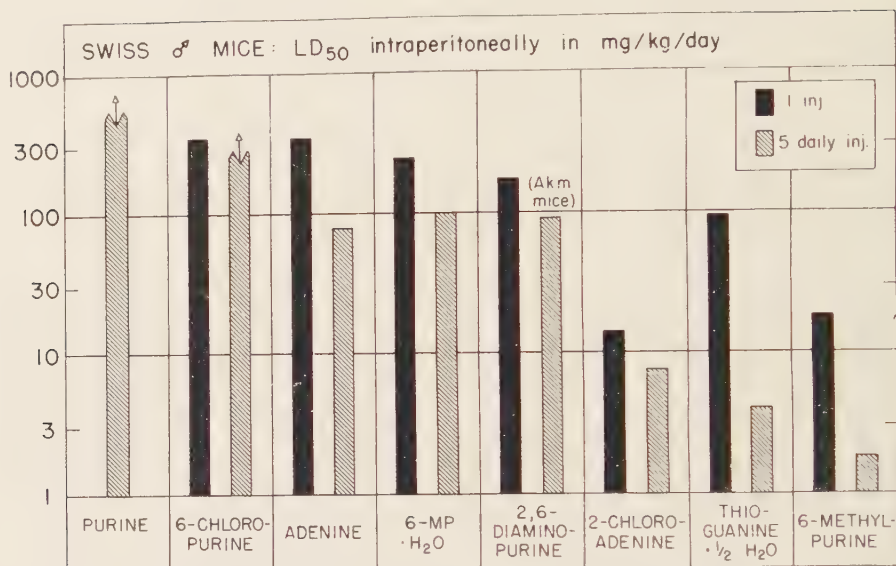


FIGURE 1. Ordinate scale in log of the LD<sub>50</sub>. All compounds administered in isotonic saline in which purine and 6-methylpurine are soluble; 6-chloropurine, adenine<sup>5</sup> and 2,6-DAP<sup>4</sup> solubilized by warming after addition of 1 mole equivalent of NaHCO<sub>3</sub>, lactic acid or HCl, and lactic acid, respectively; 6-MP suspended uniformly;<sup>6</sup> 2-chloroadenine and thioguanine suspended by dissolving with NaOH and precipitating with an equivalent amount of HCl.

purine. On the other hand, the two most toxic purines, thioguanine and 6-methylpurine, show marked enhancement of potency when given in repeated doses; expressing the relation between 5-dose and single dose LD<sub>50</sub>, as before, gives ratios of less than 1:20 and about 1:10, respectively. Indeed, this toxicologic property resembles the enhancement of potency seen during chronic administration of both Aminopterin and A-Methopterin.<sup>1</sup>

FIGURE 2 depicts similar observations in the male Wistar rat. The results are like those obtained above with mice, namely, the arrangement of the series in order of potency, the hundredfold difference in toxicity exhibited by the extreme members of the series, and the highly cumulative toxicity of multiple doses of thioguanine and 6-methylpurine.

Finally, TABLE 1 presents a comparison of toxicity of selected members of the series in dogs. The values shown are estimates based on previous reports<sup>1, 6, 7</sup> or on current, unpublished observations.<sup>9</sup> It is interesting to note that in this species the toxicity of 6-chloropurine resembles that of 2,6-DAP and 6-MP. On the other hand, in mice and rats the last mentioned substances are at least three times more potent than 6-chloropurine by chronic administration. It may again be seen that, as in mice and rats, thioguanine and 6-methylpurine are distinctly more toxic than the other purines studied.

*Acute effects.* Single intraperitoneal doses of 250 or 500 mgm./kg. of adenine<sup>5</sup> or 2,6-DAP<sup>4</sup> in mice or rats and similar doses of thioguanine<sup>9</sup> in mice produce ataxia, weakness, and dyspnea within the first few hours after injection. These changes progress in severity, and the animals die within less

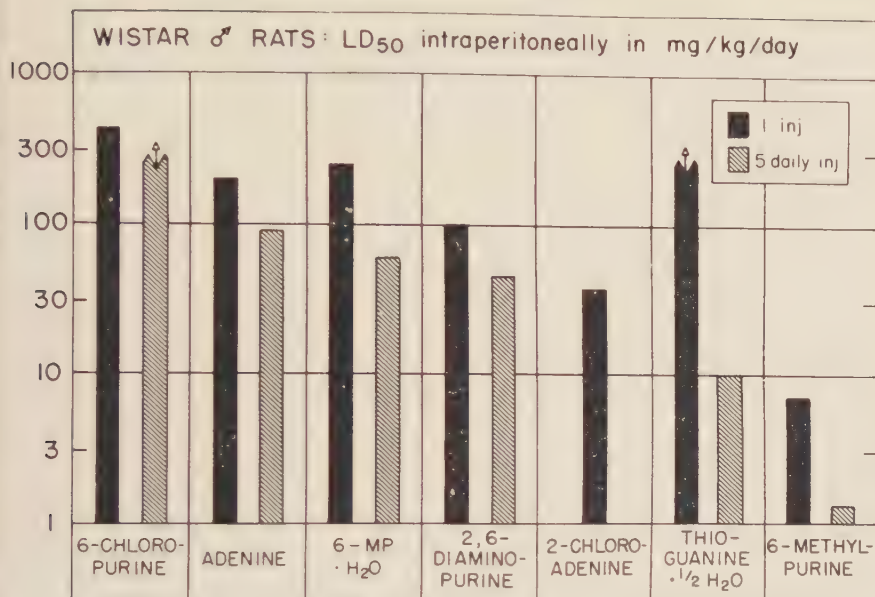


FIGURE 2. (Continued)

than 12 hours, probably as the result of respiratory failure. The overt manifestations produced by adenine have been described as a "shocklike" state and have been compared with the potent cardiovascular effects of the vaso-depressor adenine nucleosides and nucleotides.<sup>8</sup>

A second type of acute manifestation has been observed in mice or rats given single lethal intraperitoneal doses of 6-chloropurine.<sup>9</sup> Less than five minutes after injection of 500 mgm. kg. clonic convulsions develop that are terminated by death within one hour. The prior administration of 50 or 100 mgm. kg. of phenobarbital sodium in mice protects against convulsions, and such animals survive without showing delayed toxic effects. Cats given 6-chloropurine in intravenous doses of 150 mgm. kg. also exhibit transient convulsant episodes within five minutes after injection.<sup>9</sup>

TABLE I  
TOXICITY OF VARIOUS PURINES GIVEN INTRAVENOUSLY IN DOGS

Compound*	LD <sub>50</sub> <sup>†</sup>
	mgm./kg./day
2,6-DAP.....	20 to 40
6-Chloropurine.....	12.5 to 25
6-MP · H <sub>2</sub> O.....	10 to 25
Thioguanine · 1/2 H <sub>2</sub> O.....	0.6
6-Methylpurine.....	0.35

\* All compounds administered within one-half hour after dissolving in isotonic saline; 2,6-DAP solubilized by warming after addition of 1 mole equivalent of lactic acid<sup>4</sup>; 6-chloropurine, by addition of one mole equivalent of NaHCO<sub>3</sub> and warming; 6-MP · H<sub>2</sub>O<sup>5</sup> and thioguanine, by addition of 1 mole equivalent of NaOH.

† Daily dose fatal to one-half of animals receiving ten successive daily injections except during week end.



The above acute changes have not been observed in rats or mice given supralethal doses (500 mgm./kg., intraperitoneally) of 6-MP or of 2-chloroadenine. Experience with purine and 6-methylpurine has been too limited to provide information concerning this aspect of their toxicity.

*The "adenine kidney".* Toxic doses of three of the purines listed in FIGURES 1 and 2, adenine, purine, and 2-chloroadenine, result in the formation of obstructive crystalline deposits in renal tubules. The deposits produce internal hydronephrosis and inflammatory responses. In the case of adenine this effect has long been known.<sup>10, 11, 12</sup> Recent studies have confirmed an early report<sup>11</sup> that the intratubular deposits consist of an oxidation product, 2,8-dioxyadenine.<sup>5, 13</sup> The same end-product accumulates in kidney tubules after administration of either isoguanine (2-hydroxyadenine) or 8-hydroxyadenine.<sup>13</sup> Renal deposition of the oxidized purine may be attributable both to its unusually low solubility (30 times less than that of uric acid) and to its direct oxidation by tubular epithelium.<sup>5</sup> Grossly the "adenine kidney" in the rat is characterized by its large size, mottled appearance, edematous and soft consistency, and the presence of white bands or streaks in medullary tissue that appear to radiate to the papilla (see FIGURE 3). Somewhat similar alterations have been described in dogs.<sup>14</sup>

The intratubular crystals produced by giving 2-chloroadenine or purine to rats<sup>9</sup> and those caused by adenine can be distinguished microscopically by differences in morphology, color, and birefringence. On the other hand, the crystals induced by 2-chloroadenine resemble structurally and optically those found in rats or mice given 2-chloro-8-hydroxyadenine. The latter has been tentatively identified by UV absorption spectra in extracts of kidneys of rats given 2-chloroadenine. In the case of purine some, though not all, of the intratubular deposits resemble microscopically the "amorphous urates" seen occasionally in humans; but whether the crystals found after purine administration are urates is not known. Nevertheless, this is a reasonable possibility since purine is rapidly oxidized *in vitro* to uric acid by xanthine oxidase.<sup>15</sup> The same enzyme also oxidizes adenine into 2,8-dioxyadenine.<sup>16</sup>

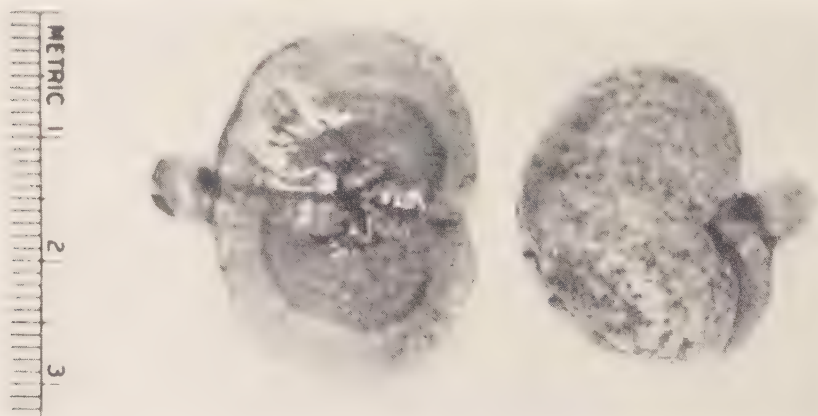


FIGURE 3. "Adenine kidney" in the rat. Specimen obtained in previous studies.<sup>5, 13</sup>

In both rats<sup>5</sup> and dogs,<sup>17</sup> as well as in man,<sup>18</sup> adenine intoxication gives rise to severe renal insufficiency. In view of this it has been difficult to accept a recent suggestion that adenine can cause a state resembling "multiple avitaminosis" in animals by interference with functions of adenine-containing coenzymes.<sup>11</sup> It seems more reasonable to attribute the syndrome to toxic manifestations of uremia.<sup>6</sup> Moreover, the known capacity of adenine to promote granulocytosis in experimental animals<sup>17</sup> and to inhibit erythrogenesis<sup>6</sup> are also considered the indirect result of renal trauma and inflammation.<sup>5</sup>

The primary effect of 2-chloroadenine and its 8-hydroxy derivative in mice and rats appears to be renal damage though granulocytosis is also observed. On the other hand, though renal obstruction and granulocytosis are prominent in rats given purine, they are not the only alterations encountered (see below).

In view of possible relationships between *in vivo* oxidation and renal crystals, it is of interest to note the *in vitro* formation of 6-thiouric acid from 6-MP by the action of xanthine oxidase.<sup>20</sup> In addition, substantial amounts of thiouric acid are produced from 6-MP *in vivo* in mice<sup>21</sup> and man.<sup>22</sup> Nevertheless, kidney crystals have not been observed in experimental animals given 6-MP.

*Pathologic effects of 6-MP in mice and rats.* As mentioned previously, overt manifestations of 6-MP intoxication are delayed. After single lethal doses the majority of rats survive for two to three days; in mice, fatalities are not encountered until at least five days after injection (FIGURE 4). The more rapid course in rats has been attributed to a species-specific pulmonary disturbance manifested outwardly by the development of dyspnea, which progresses in severity until death. At autopsy the thoracic spaces are filled with a clear

#### TOXICITY OF SINGLE, ip, DOSES OF 6-MP

	$\frac{\text{mg.}}{\text{kg.}}$	DAY OF DEATH							Mort. %	LD <sub>50</sub>
		1	2	3	4	5-7	8-14			
MICE	500					5	5		100	240
	250					2	2		55	
	125								3	
RATS	500	2	2	1		1			100	250
	250	2	1	1		1	1		50	
	125						1		8	

■ = 5 Mice or 1 Rat

FIGURE 4. Fate of male Swiss mice and male Wistar rats given 6-MP·H<sub>2</sub>O.

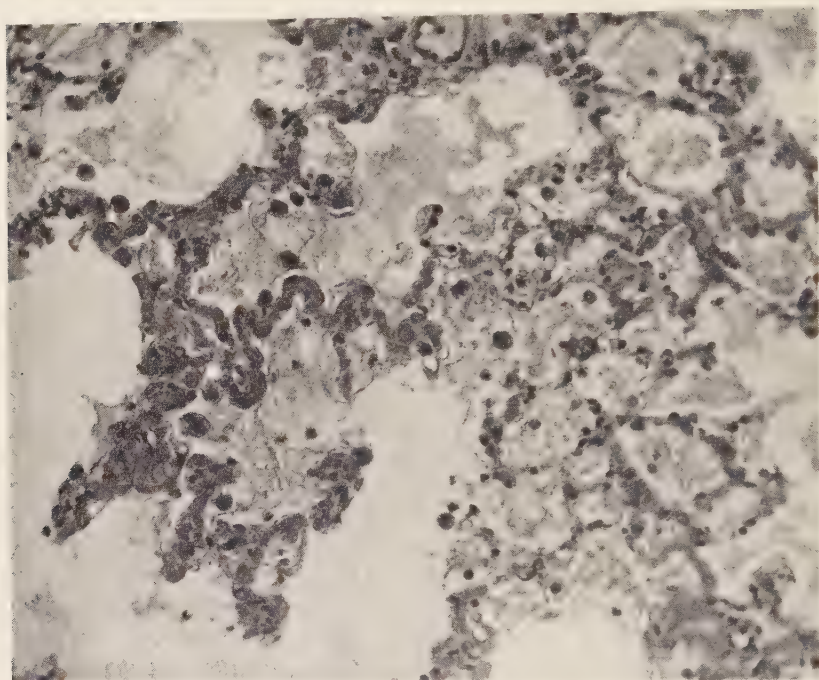


FIGURE 5. Alveolar septa thrombosed and thickened by an eosinophilic coagulum. In right half of figure septa are only slightly affected. Edema fluid and fibrin in alveoli. Male Wistar rat, 297 gm., given 6-MP•H<sub>2</sub>O, 500 mgm./kg. by oral intubation, and sacrificed at 72 hours.

fluid containing protein.<sup>6</sup> These pleural effusions are the most consistent pathologic findings, while thrombosis of alveolar septa, pulmonary edema, and hemorrhage are less frequent and, usually, confined to animals sacrificed in the later stages of intoxication (FIGURES 5 and 6). Other changes include edema of structures in the anterior mediastinum and of the hilus of the lung and focal myocarditis (FIGURE 7).

The changes just described have been observed only in the Wistar rat and have not been encountered in mice, cats, or dogs.<sup>6, 9</sup> Moreover, alveolar thrombosis and pulmonary edema have not been seen in rats receiving the other purines under consideration.

Peritonitis, in association with ascites and fibrinous adhesions among abdominal viscera, is another finding in Wistar rats given intraperitoneal doses of 6-MP. This effect appears to be a manifestation of direct damage to the peritoneum, since it is not seen in animals receiving lethal doses by oral intubation. The same lesion has been described following intraperitoneal injections of adenine<sup>5</sup> and 2,6-DAP<sup>1</sup> and has also been seen with 2-chloroadenine, thioguanine, and 6-methylpurine.<sup>9</sup>

With the above exceptions, the pathologic effects of 6-MP in mice and rats are similar. They include hepatic necrosis (FIGURE 8), lesions in intestinal epithelium, and depletion of bone marrow.<sup>6</sup> The hepatic change is specific



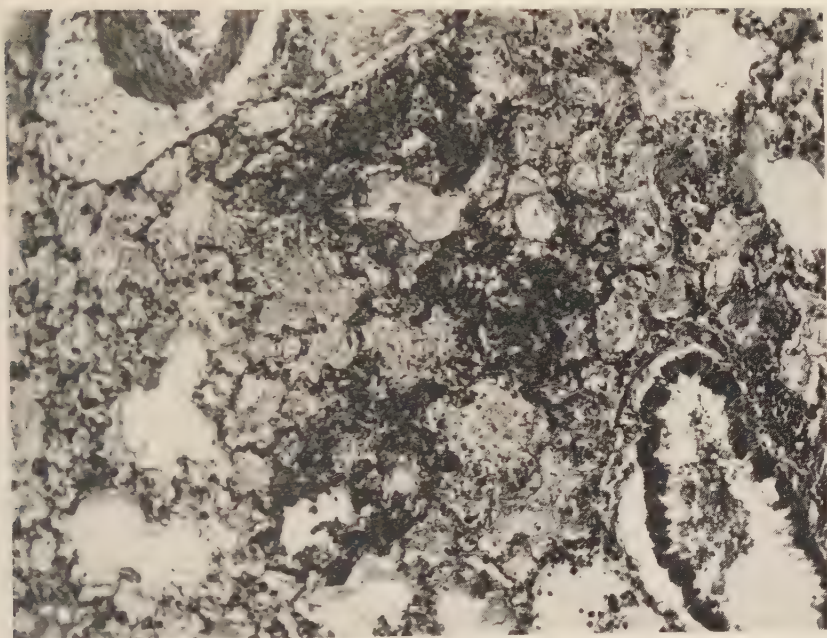


FIGURE 6. Recent hemorrhage surrounding thrombosed septa. Same rat as described in FIGURE 5.

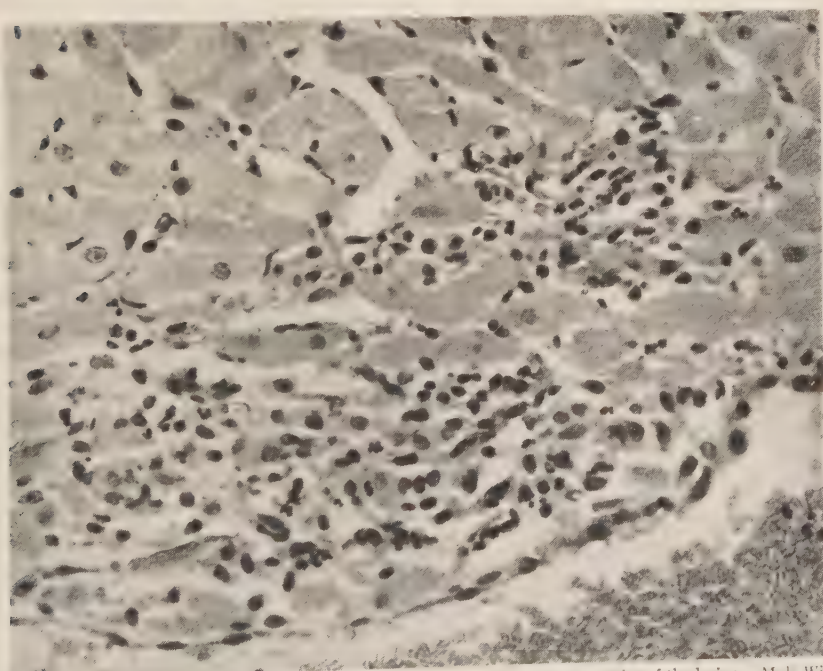


FIGURE 7. Focus of myocarditis in subendocardial region, the most frequent site of the lesion. Male Wistar rat, 230 gm., treatment and sacrifice as in FIGURE 5.



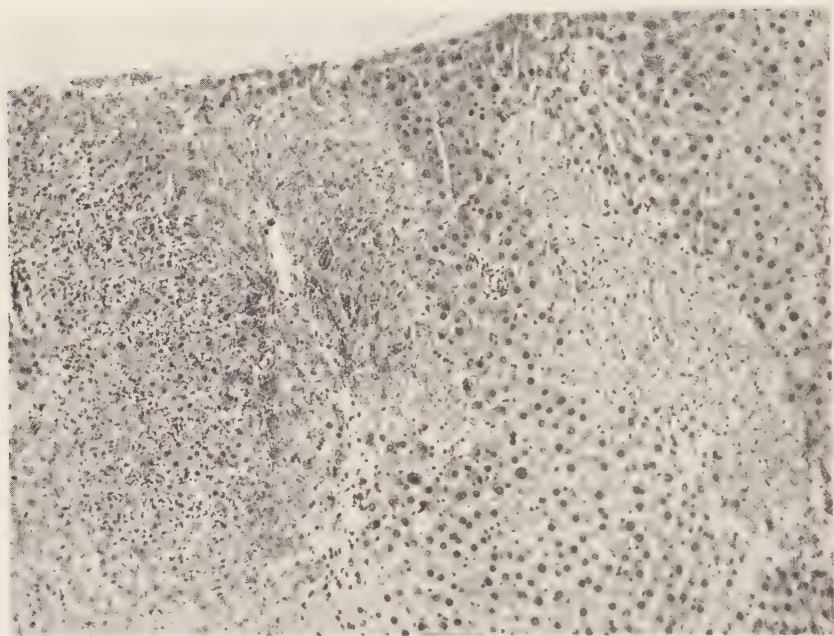


FIGURE 8. Area of acute, hepatic necrosis near surface of liver, the region in which the lesion is usually seen. Necrosis involved large areas of all liver lobes and all portions of affected lobules. Same rat as described in FIGURE 5.

for 6-MP; the other purines do not produce liver necrosis, though 2,6-DAP has been reported to induce fatty metamorphosis in pigs.<sup>23</sup> On the other hand, the changes encountered in the small and large intestines, such as epithelial atypia and edema, congestion, and leukocytic infiltration of mucosa, are also produced by 2,6-DAP.<sup>4, 24</sup> In addition, 2,6-DAP is like 6-MP in causing bone marrow hypoplasia.<sup>4</sup> The same lesion is prominent in pigs receiving 2,6-DAP.<sup>23</sup>

*Pathologic effects of 6-MP in dogs.* The toxicity of 6-MP in dogs may be seen in the data of TABLE 2. The drug appears about equally active whether given intravenously or *per os*, and the clinical course is similar by either route. All doses used in TABLE 2 produce anorexia and loss of weight. Diarrhea is a prominent feature, and it often becomes hemorrhagic in animals given fatal doses. Jaundice is another prominent effect. As shown in FIGURE 9, elevation of hematocrit and hypochloremia occur, and these effects may be attributed to diarrhea and dehydration. In addition, FIGURE 9 reveals development of hyperbilirubinemia and an associated increase in bromsulfalein retention. Leukopenia is also evident.

The jaundice and dye retention are manifestations of hepatic dysfunction which might have been anticipated, since 6-MP causes liver necrosis in mice and rats. However, livers taken from dogs jaundiced at time of sacrifice are grossly normal and reveal at most only occasional and relatively insignificant microscopic foci of necrosis. On the other hand, many intrahepatic bile ducts

TABLE 2  
TOXICITY OF 6-MP IN DOGS

Dose*	Number of injections	Route	Mortality	Day of death
<i>mgm., kg./day</i>				
50	3	IV†	2/2	4, 4**
25	4	IV	1/4	7**
10	9	IV	0/4	
25	4-8	PO††	2/2	7**, 13
12.5	7-9	PO	4/4	9, 10, 10, 13
6.3	10	PO	0/4	

\* Given on successive days except during week end.

† IV = intravenous, dissolved in isotonic saline by addition of alkali.

\*\* Sacrificed in extremis.

†† PO = per os, weighed amounts in gelatin capsules.

are found distended by inspissated bile, some of which appears calcified. Bile canaliculi are also prominent and are filled with bile. This alteration may be important in the pathogenesis of the jaundice since 6-chloropurine also causes hyperbilirubinemia and the same microscopic change in the intrahepatic biliary system. Moreover, this purine does not induce hepatic necrosis. It should be noted that the extrahepatic biliary system is normal.<sup>9</sup> The intrahepatic biliary obstruction may reflect some drug-induced change in the composition of biliary secretions. However, dehydration or impaired hepatic circulation caused by dehydration or both may be contributory.\*

The hemorrhagic diarrhea developing in dogs given fatal doses of 6-MP is associated with intestinal lesions. In the small intestine these include widespread denudation of surface epithelium, congested and dilated capillaries in tips of denuded villi, swelling and atypia of glandular nuclei, leukocytic infiltration throughout the mucosa, and regenerative changes in surface epithelium (FIGURE 10). Changes observed in large intestine are less severe. Similar lesions have been observed in animals receiving fatal doses of 2,6-DAP<sup>1</sup> and 6-chloropurine.<sup>9</sup>

Neutropenia, as shown in FIGURE 9, results from the cytotoxic effects of 6-MP in bone marrow. Previous reports described toxic changes and necrosis in nucleated erythroid and myeloid elements in marrow aspirations as early as 48 hours after initiation of treatment.<sup>6</sup> These changes presage hypoplasia, as depicted in FIGURE 11. As in the case of intestinal lesions, both 2,6-DAP<sup>1</sup> and 6-chloropurine<sup>9</sup> act like 6-MP in depleting dog-bone marrow.

*Thioguanine.* In common with 6-MP, 2,6-DAP, and 6-chloropurine, thioguanine causes depletion of bone marrow. Unlike these compounds, however, the pathologic changes of thioguanine toxicity are virtually limited to the bone marrow. Fatal doses in rodents and dogs cause minimal or no discernible microscopic alterations in intestinal epithelium; diarrhea and dehydration do not occur. During chronic administration of lethal doses in dogs, marrow aspirations reveal an early destruction of myeloid and nucleated erythroid elements; eventually there remains only a scanty population of

\* The authors are indebted to Doctor Arthur C. Allen for assistance in the interpretation of the hepatic changes in dogs.

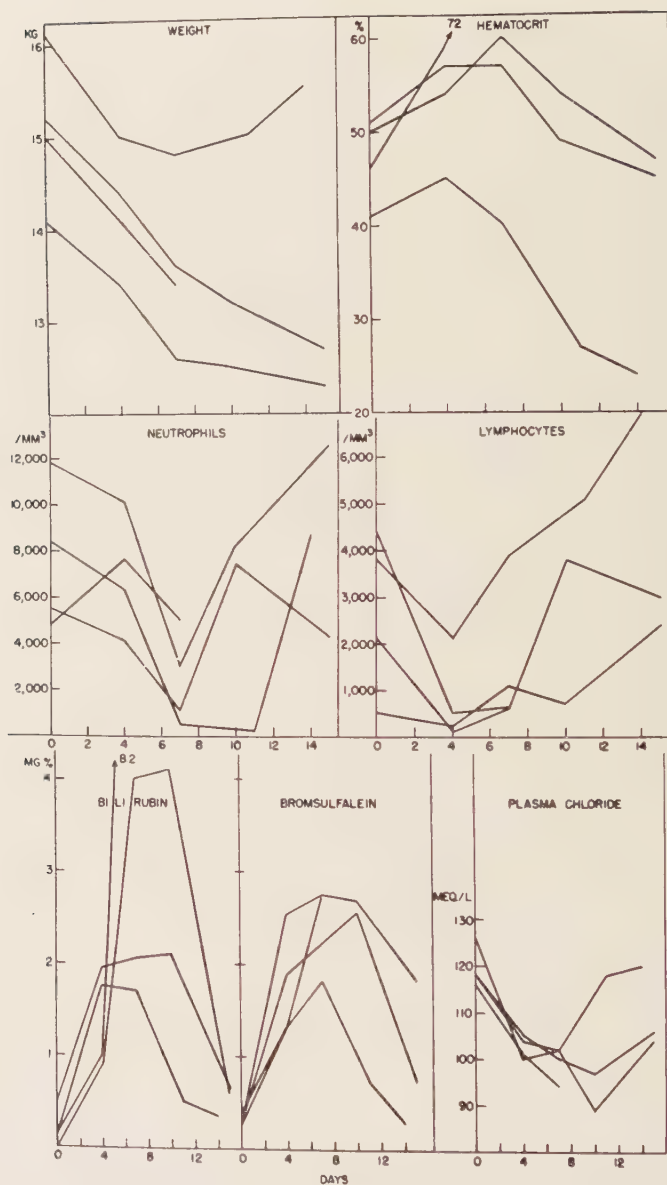


FIGURE 9. Course of intoxication in four dogs given 6-MP·H<sub>2</sub>O intravenously, 25 mgm./kg./day at 0, 1, 2, and 3 days. One dog, with a 72 per cent hematocrit, was sacrificed at 7 days; the other three survived.

primitive reticulum and plasma cells. A progressive reticulocytopenia, anemia, thrombocytopenia, and granulocytopenia ensue. After agranulocytosis develops, dogs invariably become febrile several days before death and sometimes exhibit marked pharyngitis. Presumably the fevers are septicemic



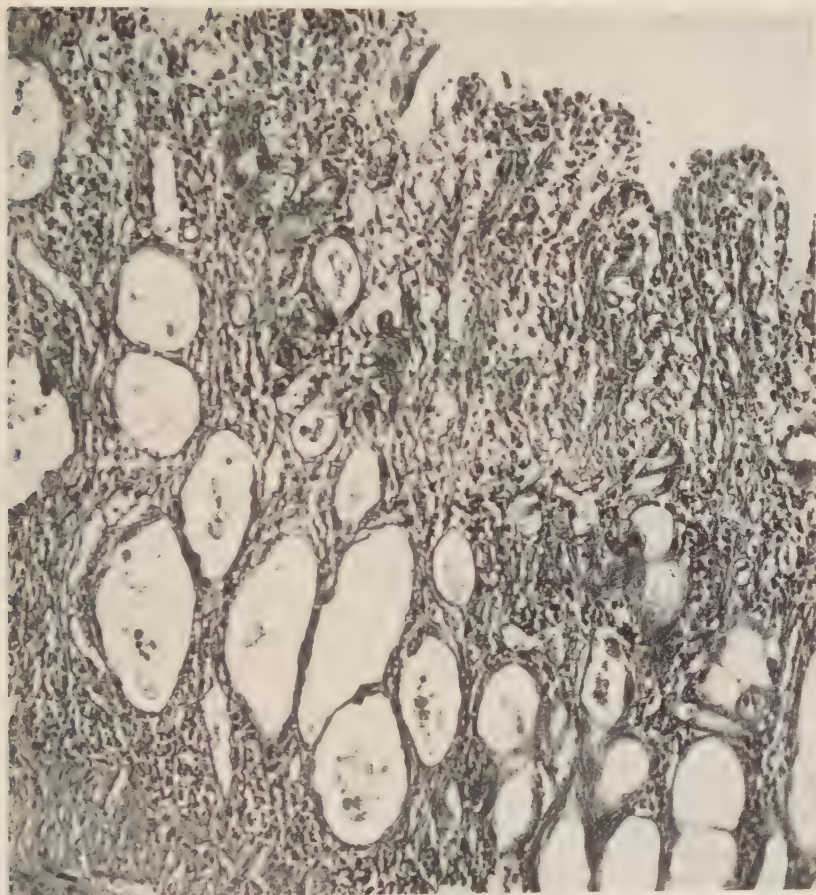


FIGURE 10. Ileum of sacrificed dog, described in FIGURE 9, showing ectasia of glands, epithelial atypia, denuded villi, and inflammatory infiltrate

in origin. By the use of antibiotics, body temperatures can be reduced and maintained at normal levels for a time; but animals so treated eventually succumb with generalized hemorrhage. This effect is foreshadowed by progressive increases in blood clotting time.<sup>7</sup>

The fatal effects of thioguanine appear to result from agranulocytosis or thrombocytopenia or both. Insofar as these changes are concerned, the clinical manifestations and the protracted course of intoxication in dogs resemble the effects of LD<sub>50</sub> doses of ionizing radiation.<sup>26</sup> Nevertheless, thioguanine can be differentiated from radiation poisoning by the fact that lesions in lymphoid tissues and intestinal epithelium are not prominent.

*Purine and 6-methylpurine.* Studies in progress have revealed these substances—the extreme members of the present series in regard to toxicity—to be of unusual interest because of wholly unanticipated effects. In addition to



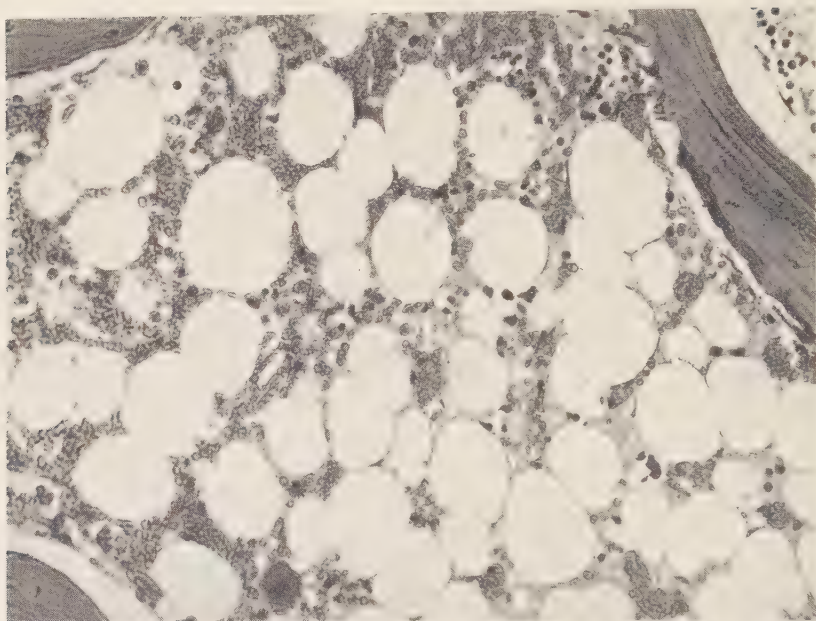


FIGURE 11. Vertebrum with marked depletion of hematopoietic cells. The sinusoids are predominantly occupied by mature red cells rather than nucleated elements. Dog given 6-MP·H<sub>2</sub>O by mouth, 25 mgm./kg./day at 0, 1, 2, and 3 days; sacrificed in extremis at 7 days.

the kidney crystals described above, purine causes in rats karyorrhectic changes in lymphoid follicles of thymus, nodes, and spleen, in bone marrow, in glandular epithelium of stomach and intestine, and in stromal elements of intestinal mucosa. Diffuse mucosal congestion is prominent in rat intestine and is associated with focal hemorrhages and with suppurative erosions and ulcerations. The gastrointestinal effects are distinguished from those described previously by the absence of typical mercaptopurine-like atypia in epithelial cells, by the involvement of the lamina propria, and by the presence of lesions in the glandular stomach. Lymphoid karyorrhexis is another novel change which has not been observed previously as a prominent effect of toxic purines. Finally, though purine causes some depletion of bone marrow and moderate reticulocytopenia, leucopenia is not observed; indeed, the reverse occurs, namely, a significant granulocytosis (see above).

The effects of 6-methylpurine are even more unexpected. This highly toxic purine, when administered in minimum lethal doses to rats or dogs, causes a greatly protracted intoxication characterized by progressive weight loss and anorexia (observation in dogs). Persistent anorexia and cachexia remain unexplained; the dogs do not develop diarrhea, and tests of renal and hepatic function have been uninformative.

Although reticulocytopenia is prominent and aspirations of bone marrow reveal deficiencies of normoblasts, leucopenia and thrombocytopenia are not seen in intoxicated dogs. In the poisoned rat, hypoplasia of bone marrow is at most minimal, and granulocytosis is marked.

Microscopic study has also failed to demonstrate lesions in the intestinal mucosa of rats and dogs given one to two times the multiple-dose LD<sub>50</sub>. Indeed, histological studies have been singularly uninformative regarding the nature of the intoxication in dogs. In rats, in addition to ascites and pleural effusions, edema is seen in various sites such as testis, prostate, wall of bladder, thyroid, hilus of lung, perirenal and periadrenal tissues, and capsule of salivary glands. It is unlikely that the generalized edema is attributable to losses of plasma protein, for its early appearance contravenes starvation as a cause, and renal lesions are not observed.

*Comment.* The above study has indicated certain potential hazards in therapeutic applications of 6-MP. These effects include damage to bone marrow and intestinal epithelium, disturbances in hepatic function, and the possibility of liver necrosis. In addition, 6-MP elicits a species-specific pulmonary lesion in Wistar rats.

Comparisons of 6-MP with seven closely related purine analogs have revealed a number of common pharmacologic properties. Like adenine, purine, and probably 2-chloroadenine, 6-MP is in part oxidized *in vivo* into and excreted as a uric acid analog; in contrast to these purines, the end-product of 6-MP metabolism, 6-thiouric acid, does not crystallize in renal tubules. Accordingly renal insufficiency, which is prominent in animals given adenine or 2-chloroadenine, has not been encountered among the effects of 6-MP.

Damage to mammalian bone marrow is a second property common to 6-MP and the majority of the purines, namely, 6-chloropurine, 2,6-DAP, thioguanine, 6-methylpurine, and purine. It seems more than coincidence that this same group of bone-marrow depressing purines is inhibitory in certain rodent tumors and leukemias, as mentioned elsewhere in this symposium, and that 6-MP is effective in clinical leukemias. That clinical diseases of hematopoietic origin, such as the leukemias, are sensitive to bone-marrow depressants suggests a common mechanism of action in normal and malignant cells.<sup>26</sup>

Nevertheless, the purine analogs of 6-MP are not identical with regard to specificity of action in bone marrow. For example, 6-MP, 2,6-DAP, and 6-chloropurine produce similar lesions in intestinal epithelium when given in doses causing depression of bone marrow; the effects of thioguanine are largely limited to the bone marrow, in which most hematopoietic elements are susceptible; and 6-methylpurine appears selectively depressant to erythropoiesis. These differences in tissue susceptibility and others mentioned above may in time be related to distinguishable metabolic derangements. At present, however, terms such as "antipurines" or "antagonists of nucleic acid metabolism" fail to elucidate the selectivity of action exhibited by close structural analogs of 6-MP, for example, thioguanine and 6-methylpurine. By contrast, antagonism of folic acid metabolism is a reasonable explanation of the toxic effects of 4-amino substituted derivatives<sup>1</sup> of the vitamin or of certain 2,4-diaminopyrimidines.<sup>2, 2a</sup> Whatever mechanisms are ultimately proposed, however, it is abundantly clear at present that minor alterations in the structure of purines can give rise to profound and unexpected changes in their pharmacologic effects.

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## THE FATE OF 6-MERCAPTOPURINE IN MICE\*

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Among the reasons for undertaking the study of analogs of the purine and pyrimidine bases<sup>1-2</sup> was the possibility that such substances might be utilized for the formation of unnatural nucleic acids. The incorporation of compounds of this type into nucleic acids of mice, *Tetrahymena gelii*, tobacco mosaic virus, and bacteria has been reported recently for 8-azaguanine,<sup>3-6</sup> 5-bromouracil,<sup>7</sup> and 2-thiouracil.<sup>8</sup> When 6-mercaptopurine proved to behave like a purine antagonist in microorganisms<sup>9,10</sup> and to have such interesting biological effects,<sup>11,12</sup> it became important to determine whether this compound could be incorporated into the nucleic acids. Accordingly, two types of radioactive 6-mercaptopurine were prepared,<sup>13</sup> the one labeled in the 8-position of the purine nucleus with C<sup>14</sup>, the other labeled in the mercapto group with S<sup>35</sup>.

As a preliminary to studies in the human, an investigation of the metabolic fate of 6-mercaptopurine in the mouse was undertaken. These experiments were designed primarily to determine how long the drug remained in the body, what metabolic products were formed, whether the drug was preferentially concentrated in any particular tissues, and whether the compound was incorporated into the nucleic acids.

After the intraperitoneal injection of 1 mgm. of S<sup>35</sup>-6-mercaptopurine per mouse, the urines from 20 mice were pooled and the radioactivity determined at various time intervals. The amount of radioactive material excreted in the first four hours was 435  $\gamma$ -equivalents of S<sup>35</sup>-6-mercaptopurine† per mouse, or 43.5 per cent of the injected material. By the end of two days, over 60 per cent of the S<sup>35</sup> had been excreted (TABLE 1). Approximately the same rate of urinary excretion of radioactive material was found after oral administration of 8-C<sup>14</sup>-6-mercaptopurine. In the latter case, some radioactive carbon dioxide was also detectable in the expired air.

Paper chromatography was carried out on each of the urinary specimens, using a 5 per cent ammonium sulfate-5 per cent isopropanol system, in order to determine the distribution of radioactivity (TABLE 1). A typical distribution pattern for the chromatogram of the 26-hour urine specimen is shown in FIGURE 1. The radioactivity was found to be concentrated in three principal spots, at Rf values of 0.27, 0.39,\*\* and 0.76. The material with the low Rf value corresponds to 6-thiouric acid, which can also be formed from 6-mercaptopurine *in vitro* by treatment with xanthine oxidase.†† The unchanged 6-mercaptopurine has an Rf of 0.44; the substance with Rf 0.76 is an unidentified metabolite.

\* Supported in part by a grant to the Wellcome Research Laboratories from the Charles F. Kettering Foundation.

† Although the S<sup>35</sup>-containing material in the biological specimens is not always identifiable, it is convenient to record the activities in terms of the starting material. Because of the relatively short half life of S<sup>35</sup> (87 days) it is necessary to compare the activity of the sample to the activity of the standard on the day of counting.

\*\* This Rf value is calculated from the center of a 2-cm. strip covering the range 0.36 to 0.43. Other experiments indicate that a more accurate Rf value for 6-mercaptopurine is 0.44.

†† Unpublished data of D. Lorz.



TABLE 1  
URINARY EXCRETION AFTER I.P. INJECTION OF S<sup>35</sup>-6-MP IN THE MOUSE

Hours after injection	Total $\gamma$ -equivs. S <sup>35</sup> -6-MP per mouse	Distribution of S <sup>35</sup> , % of total*			
		6-thiouric	6-MP	Rf 0.76	Rf 0.6
0-4	435	39	32	0	4
4-26	140	26	23	37	5
26-49	33.3	20	11	54	4
49-96	16.1	16	8	5	67

\* Determined by paper chromatography.

During the first four hours the main excretory products were found to be 6-mercaptopurine and 6-thiouric acid (TABLE 1). The remainder of the radioactivity was scattered through the chromatogram, particularly near the origin. From the period 4 to 49 hours after injection, the percentage of both of these components decreased and a metabolite with Rf 0.76 became prominent. During the third and fourth days, a new metabolite with Rf 0.6 became the major component, and the percentages of the other three products all decreased. Although the material with Rf 0.6 accounted for 67 per cent of the radioactivity in the 49 to 96 hour specimen, the absolute amount of this material was very small (10.7  $\gamma$ -equivalents), since the total radioactivity of this sample was only 16.1  $\gamma$ -equivalents.

Since the efficacy of a chemotherapeutic agent is related to the concentration which can be maintained in the body over a certain period of time, it was important to determine the rate at which the concentration of the drug in the blood decreased after a single dose. Mice were injected i.p. with 1 mgm. of S<sup>35</sup>-6-mercaptopurine, and samples of blood were drawn from the tail at one-half hour intervals thereafter. Data from two individual mice (FIGURE 2) indicate that radioactivity falls rapidly during the first five hours. By the end of 25 hours, the blood level had dropped to *ca.* 0.5  $\gamma$ -equivalents per ml.

In order to determine whether 6-mercaptopurine is concentrated preferentially in any particular tissues, cold trichloroacetic acid extracts of the individual organs were prepared at intervals after intraperitoneal injection of 1 mgm. of 6-mercaptopurine per mouse. The activities given in TABLE 2 represent averages of five mice and show that the concentration of radioactive material was highest in the gut, almost twice as high as in the blood, and lowest in the brain. It would appear that, in the mouse, using intraperitoneal injection, 6-mercaptopurine has some difficulty in passing the blood-brain barrier, since the concentration in the brain is only *ca.* one tenth that in the blood during the same period.

Studies on incorporation into the nucleic acids were undertaken with several objectives in view. One was to determine whether various tissues incorporated the unnatural substance to different extents. Approximate values for the incorporation of S<sup>35</sup> into the nucleic acids of the individual tissues were obtained from the radioactivities of the hot trichloroacetic acid extracts following the procedure of Schneider<sup>14</sup> (TABLE 2). Incorporation was found to be

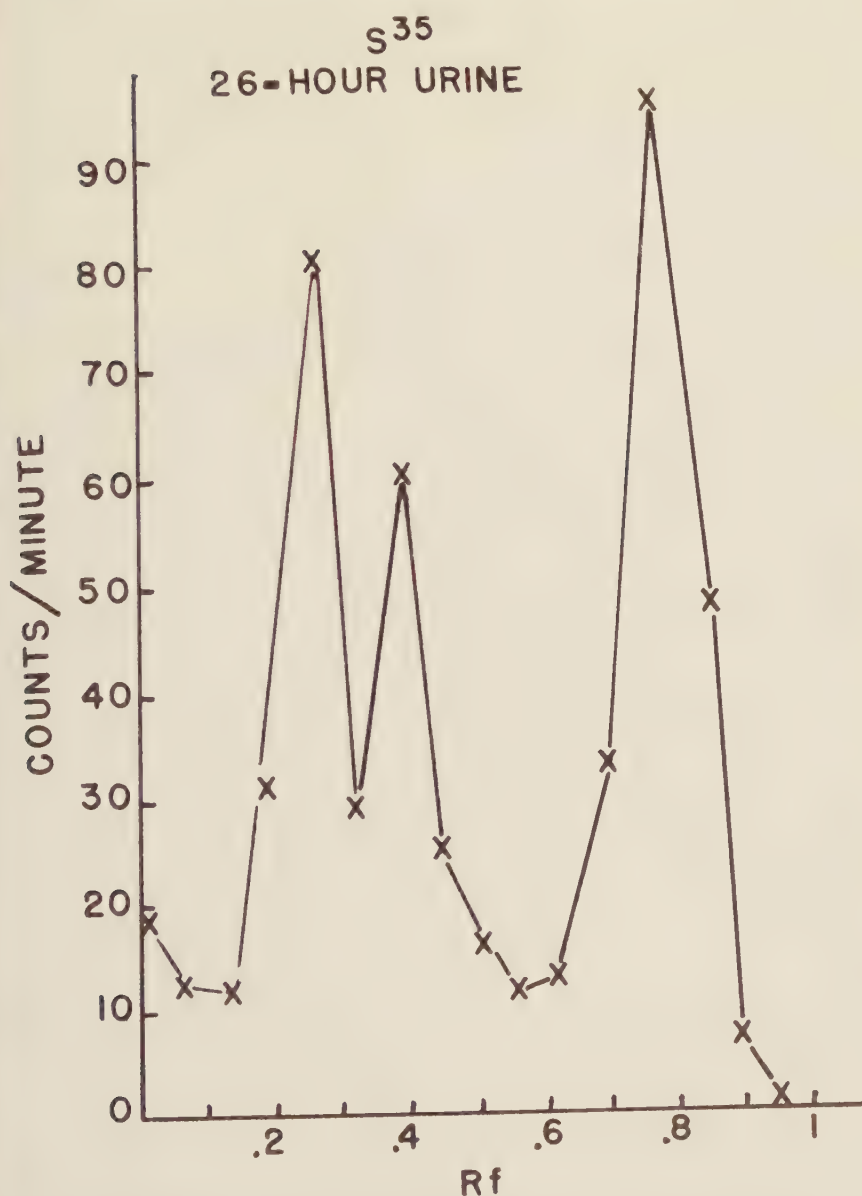


FIGURE 1. Distribution of radioactivity on a paper chromatogram of a mouse-urine sample collected 4 to 26 hours after intraperitoneal injection of  $S^{35}$ -6-mercaptopurine.

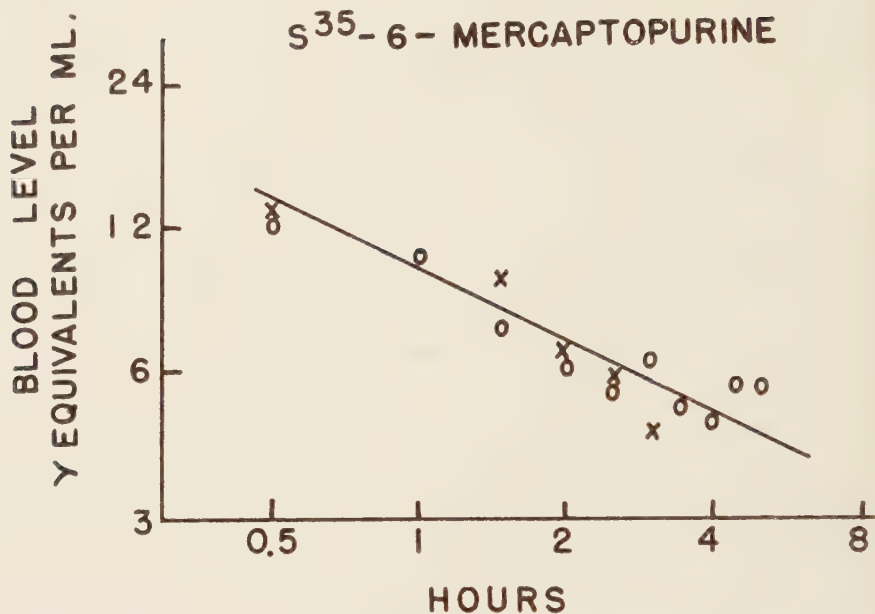


FIGURE 2. Change of S<sup>35</sup> concentration in mouse blood with time after intraperitoneal injection of S<sup>35</sup>-6-mercaptapurine.

TABLE 2  
RADIOACTIVITY OF TRICHLORACETIC ACID EXTRACTS OF MOUSE TISSUES

Tissue	γ-Equivs.-S <sup>35</sup> -6-MP per gm. wet tissue*	
	Cold TCA	Hot TCA
Liver . . . . .	5	1.1
Kidney . . . . .	7	0.7
Gut . . . . .	8	2.9
Lung . . . . .	3	0.7
Spleen . . . . .	2	0.6
Sternum . . . . .	3	0.4
Brain . . . . .	0.5	0
Blood . . . . .	4.5	

\* Average of five mice, 1½, 2, 3, 4, and 5 hours after intraperitoneal injection of 1 mgm. of S<sup>35</sup>-6-mercaptapurine per mouse.

highest in the gut, as might have been expected from the high rate of mitosis in the intestine, lower but detectable in the liver, spleen, kidney, lung, and sternum, but not detectable in the brain tissue.

Another objective was to ascertain whether the turnover rate of the unnatural component of the nucleic acid was very rapid, or whether such a constituent might remain bound even after its concentration in the blood and acid-soluble fraction had markedly decreased. In addition, it was important to determine whether the incorporated material contained both the purine

TABLE 3  
 RADIOACTIVITY OF EXTRACTS OF MOUSE TISSUES\* AFTER INTRAPERITONEAL INJECTION OF  
 RADIOACTIVE 6-MERCAPTOPURINE

Extract	$\gamma$ -Equivalents 6-MP per gm. dry tissue			
	$C^{14}$		$S^{35}$	
	3 hr.	25 hr.	3 hr.	25 hr.
Cold TCA.....	68.5	10.2	71.0	19.3
70% EtOH.....	11.0	0	1.4	1.9
EtOH-ether.....	1.2	0	0.8	0
Ether.....	0	0	0.6	0
Hot TCA.....	3.65	4.30	4.05	3.55

\* Gut, liver, spleen and kidney pooled.

skeleton and the mercapto group of the administered 6-mercaptopurine. For this purpose, pools of four tissues were examined (gut, liver, spleen, and kidney) 3 and 25 hours after intraperitoneal injection of 1 mgm. of radioactive 6-mercaptopurine. Duplicate sets of experiments were conducted, using the  $8-C^{14}$  and the  $S^{35}$  labeled material (TABLE 3). The acid-soluble fractions showed a high degree of radioactivity at the 3-hour period, which dropped markedly after 25 hours. This result was to be expected on the basis of a similar fall in the blood level during the same time interval. Small amounts of radioactivity were seen in the alcohol extracts, but little or none in the ether washings. The hot trichloroacetic extracts showed approximately the same activity after 3 and 25 hours, whether  $C^{14}$  or  $S^{35}$  labeled 6-mercaptopurine was used. This result would appear to indicate that the unnatural nucleic acid, once formed, is reasonably stable and does not depend on the concentration of the drug in the acid-soluble portion to maintain the abnormal constituent intact. The presence of  $S^{35}$  in the hot trichloroacetic acid extracts indicates only incorporation of sulfur into the nucleic acids, but does not prove that 6-mercaptopurine is incorporated as such. Paper chromatography of these hot TCA extracts, after removal of the excess TCA by ether, showed three radioactive components, only one of which had an Rf value corresponding to 6-mercaptopurine. The presence of  $C^{14}$  in the nucleic acids after administration of  $8-C^{14}$ -6-mercaptopurine indicates that the purine moiety was incorporated. There is evidence from ion-exchange chromatography of the hot TCA extract that only traces of  $C^{14}$  appear in the adenine and guanine moieties, and that most of the  $C^{14}$  activity is in a fraction which is more acidic in nature. This finding constitutes presumptive evidence that the sulfur-carbon linkage is still intact in the incorporated material.

To determine whether  $S^{35}$  is incorporated into both the ribonucleic acid (RNA) and deoxyribonucleic (DNA) acid fractions of the nucleic acids, the sodium nucleates from the four pooled tissues (gut, liver, kidney, and spleen) were isolated by extraction with hot 10 per cent sodium chloride and precipitation with alcohol, after the usual extraction with cold TCA, alcohol, and ether. The sodium nucleates were then separated into RNA and DNA, and the DNA



TABLE 4  
INCORPORATION OF S<sup>35</sup> INTO THE SODIUM NUCLEATES OF MOUSE TISSUES\*

Hours after injection	Fraction	RNA mgm./ml.	DNA mgm./ml.	M $\gamma$ -Equivs. S <sup>35</sup> 6-Mercaptopurine	
				per ml.	per mgm. N.A.
3	RNA	4.53	0.71	124	23.6
3	DNA	0.18	2.24	35	14.5
25	RNA	4.41	0.94	124	22.6
25	DNA	0.14	4.62	36	7.6

\* Liver, kidney, spleen, and gut pooled.

fraction purified according to the modification of the Schmidt-Thannhouser procedure described by Schmitz.<sup>15</sup> The amount of ribose and desoxyribose in each fraction was determined by the orcinol<sup>16</sup> and indole<sup>17</sup> reactions respectively. The total amount of nucleic acid in each fraction was calculated both from the sugar determinations and spectrophotometrically. The data (TABLE 4) show that although the RNA samples contained 12 per cent (3 hour) and 18 per cent (25 hour) of DNA, the DNA samples contained less than 8 per cent contamination by RNA. In each case, the sample is sufficiently pure so that the activity of one nucleic acid cannot be attributed to contamination by the other. It will be noted (TABLE 4) that the radioactivity of the RNA fraction is almost the same 3 hours and 25 hours after injection, in agreement with the results found with the hot trichloroacetic acid extracts. The radioactivity of the DNA fraction was lower than that of the RNA in each case, and showed a much more pronounced drop with the elapse of time, 60 per cent of the RNA value at 3 hours and 33 per cent of the RNA value at 25 hours.

Finally, an objective of these incorporation studies is the identification of the unnatural constituents of the nucleic acids. Evidence from the chromatography of the hot TCA extracts indicates that several sulfur-containing substances may have been incorporated. This is not unexpected since the formation *in vivo* of several metabolites of 6-mercaptopurine was shown by chromatography of the urine. The isolation and identification of the substances present in the nucleic acids and the interrelationship between these and the other metabolites are still under investigation.

### Summary

Studies with radioactive material have shown that 6-mercaptopurine is rapidly metabolized in the mouse with the excretion of 6-thiouric acid and several unknown products.

The concentration of radioactivity during the first few hours after intraperitoneal injection is highest in the gut and very low in the brain. This finding is also true with regard to incorporation into the nucleic acids of the individual tissues.

Three hours after injection of S<sup>35</sup>-6-mercaptopurine, radioactive material has been incorporated into both the RNA and DNA fractions of the pooled

tissues. After 25 hours, the radioactivity in the RNA was not decreased appreciably, whereas that in the DNA has dropped to about half of the 3-hour value.

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## THE FATE OF 6-MERCAPTOPURINE IN MAN\*

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6-Mercaptopurine induces temporary remissions in acute leukemia of both children and adults, and also ameliorates patients afflicted with chronic myeloid leukemia.<sup>1</sup> These effects led to a study of its distribution and metabolism in man. Such information might be of value in bettering existing therapeutic techniques, might contribute to understanding the mechanism of the action of 6-MP, and might guide the development of more active agents. For this purpose, 6-MP, labeled in the 6-position with sulfur 35, was given to a child with acute stem-cell leukemia and to an adult with chronic myeloid leukemia. The present communication describes the results, which were essentially similar in the child and the adult.

*Experimental.* FIGURE 1 summarizes the clinical course of the 2-year-old 14.1-kilogram female child with acute stem-cell leukemia who received intravenously 88 mgm. of 6-mercaptopurine-6-S<sup>35</sup> with approximate radioactivity of 100 microcuries—a dose equivalent to 6 mgm./kg. The child had been receiving unlabeled 6-MP, 37.5 mgm./day, *i. e.*, 2.5 mgm./kg./day, for about 130 days previously. In addition, for approximately the last 90 days, the child had been receiving 25 mgm./day of O-diazoacetyl-L-serine. No 6-MP or O-diazoacetyl-L-serine was given the child on the day before giving the labeled 6-MP. She was again given 2.5 mgm./kg./day of 6-MP and 2 mgm./kg./day of O-diazoacetyl-L-serine on day 5 and continued on these agents for some 30 days thereafter.

For three to four weeks before the experiment, the child, whose bone marrow was in relatively good state—22 per cent stem cells, 30 per cent myeloid, 8 per cent lymphoid, and 40 per cent erythroid—had signs of leukemic involvement of the meninges and brain; she was irritable and drowsy with bilateral papilledema and slight right facial weakness. Lumbar puncture revealed an elevated pressure and a cell count of 990, of which 98 per cent were mononuclears. The cerebrospinal fluid protein was likewise elevated to 68 mgm./100 ml. The question whether 6-MP crossed the blood-brain barrier was raised, because a similar clinical picture of meningeal involvement by the leukemic process had been seen in several patients in whom the blood picture suggested adequate hematological control following treatment with 6-MP.

The labeled 6-MP was dissolved immediately before use with 1N NaOH, which was diluted with 0.9 per cent solution of NaCl and injected over a one-minute period into an intravenous infusion of 0.9 per cent solution of NaCl. Blood samples were drawn at 5, 10, and 30 minutes, 1, 9, and 29 days thereafter. Cerebrospinal fluid samples were drawn at 5 and 30 minutes, and at 1 and 9 days. It had been intended to collect all urine voided by the child for a 48-hour period. The first two hours collection, unfortunately, was lost.

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Urine collections were thus made at 3-5, 5-7 $\frac{1}{2}$ , 7 $\frac{1}{2}$ -8 $\frac{1}{2}$ , 8 $\frac{1}{2}$ -22 $\frac{1}{4}$ , 22 $\frac{1}{4}$ -33 $\frac{1}{2}$ , 33 $\frac{1}{2}$ -34, 34-45 $\frac{1}{2}$ , 45 $\frac{1}{2}$ -48 $\frac{1}{2}$  hours. A further urine specimen was obtained on day 29.

FIGURE 2 summarizes part of the clinical course of a 46-year-old 75-kilogram male adult afflicted with chronic myeloid leukemia who received intravenously 450 mgm. of 6-MP-6-S<sup>35</sup> with approximate radioactivity of 200 microcuries. Again this dose was one of about 6 mgm./kg. The patient's illness became obvious in January 1952, and he had received several courses of X-ray therapy to the spleen, his last treatment being administered two months before the experimental period.

Except for transfusions given before the experiment, and on days 15 and 16, he remained untreated until day 17, at which time he received 5 mgm./kg. of 6-MP daily for 10 successive days.

The labeled 6-MP was given as in the child. Blood samples were drawn at 3, 35, 40, 60, 120, and 300 minutes, and on days 1, 2, 3, 4, 6, 8, and 13. Cerebrospinal fluid was drawn at 30 and 60 minutes. Urine was collected at 1, 2, 3, 4, 5, and 24 hours, and at 24 hourly intervals thereafter for 45 days. Urea clearance determination was made between hours 2 and 3. Twenty-four hour specimens of feces were collected at 24 and 48 hours.

Blood samples from the child were drawn into sodium citrate and from the adult into either sodium citrate or ACD solution. Aliquots of whole blood,

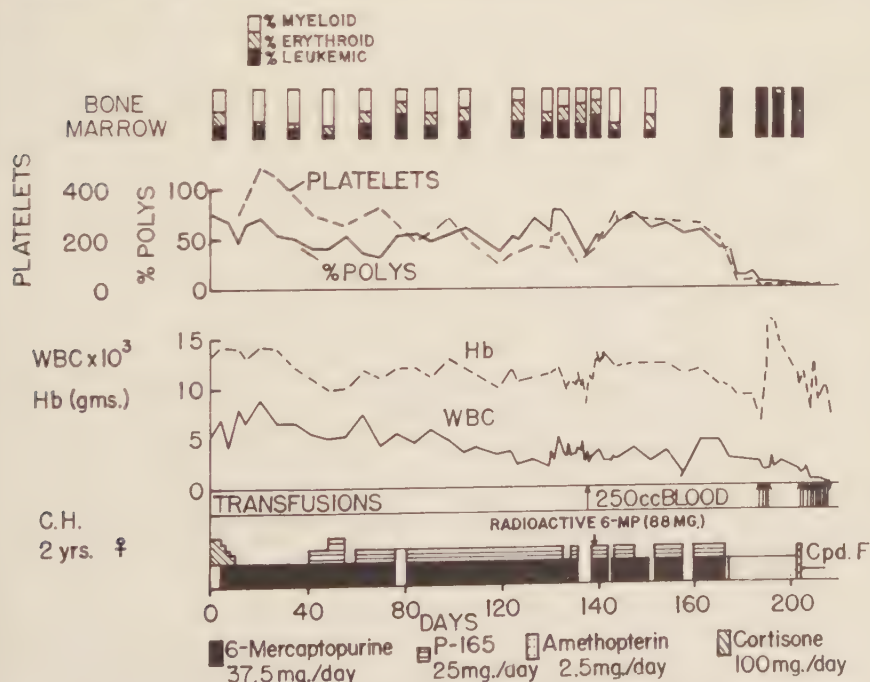


FIGURE 1. The clinical course of the 2-year-old 14.1-kilogram female child with acute stem-cell leukemia given intravenously 88 mgm. 6-MP-6-S<sup>35</sup> (100 microcuries)—6 mgm./kg.



plasma, cerebrospinal fluid, urine, and extracts of feces were evaporated to dryness on weighed aluminum planchets, and the radioactivities were measured in an internal Geiger-Muller flow counter (Radiation Counter Laboratories Counter Mark 13, Model 3. helium-isobutane mixture). The samples were counted to within 3 per cent error. The counts obtained were then corrected for activity absorbed by sample thickness.

Samples of plasma and cerebrospinal fluid were chromatogrammed on paper in isopropanol- $(\text{NH}_4)_2\text{SO}_4$  and isoamyl alcohol- $\text{Na}_2\text{HPO}_4$  media. Samples of urine were chromatogrammed on paper in isopropanol- $(\text{NH}_4)_2\text{SO}_4$  medium; samples with the greatest amount of radioactivity and the largest number of metabolites were subjected to anion-exchange chromatography on Dowex-1. The Dowex-1 fractions, after treatment with uricase, were chromatogrammed on Dowex-50, and the resultant fractions paper-chromatogrammed in isopropanol- $(\text{NH}_4)_2\text{SO}_4$ . The identity of the radioactive metabolites separated in

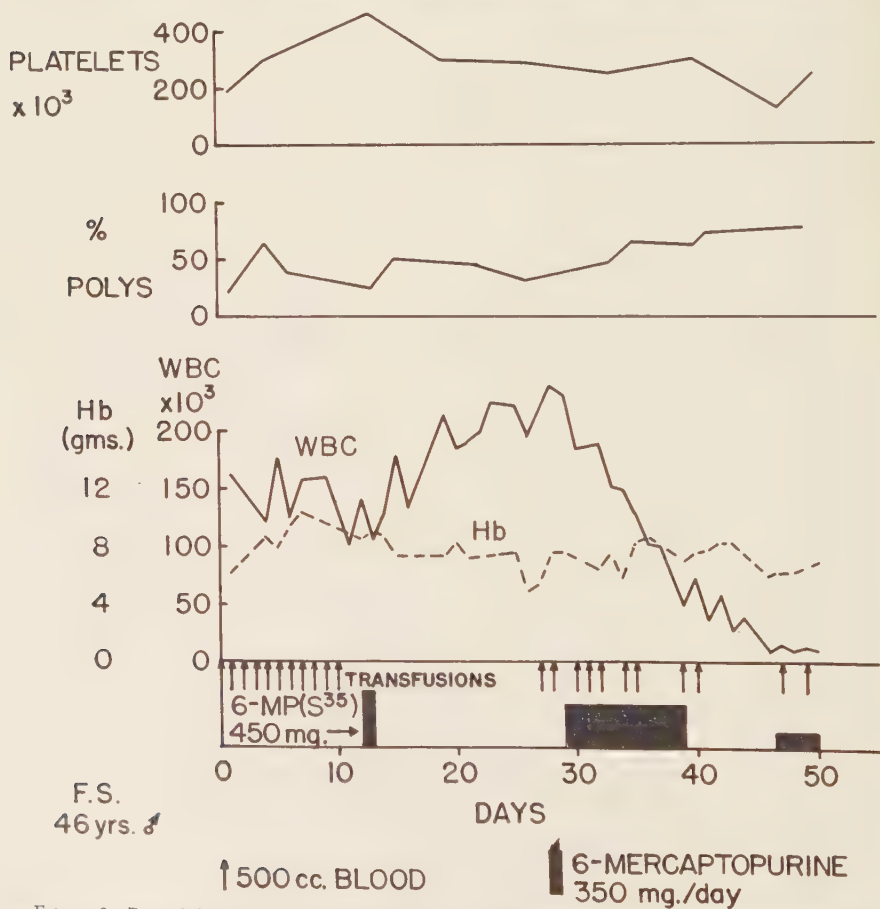


FIGURE 2. Part of the clinical course of the 46-year-old 75-kilogram male adult with chronic myeloid leukemia given intravenously 450 mgm. 6-MP-6-S<sup>35</sup> (200 microcuries)—6 mgm./kg.

this way was determined, where possible, by Rf and spectrophotometrically; activity was measured in an internal Geiger-Muller flow counter (Tracer Laboratories SC16, helium-isobutane mixture). A more detailed account of the methods used in this investigation will be published elsewhere.<sup>2</sup>

**Results.** The results obtained in the child and the adult were similar and demonstrated a rapid metabolism of the drug. FIGURE 3 shows the decline in activity in the blood of the adult. Radioactivity expressed in thousands of counts min./ml. of whole blood and plasma is plotted against time (expressed in minutes, hours, and days) on a logarithmic scale after the injection of 6-MP. As in the mouse, the radioactivity followed a normal die-away curve. Activity in the plasma declines below that in whole blood so that, eventually, when there is no significant activity in the plasma, there is still radioactivity in the whole blood, presumably associated with blood cells. The same relation between whole blood and plasma was seen in the child. On days 9 and 29, when there was no significant activity in the plasma, activities in the whole blood were accordingly 1170 and 910 counts/min./ml., respectively. The activity of the labeled 6-MP given the child was approximately 2.6 times

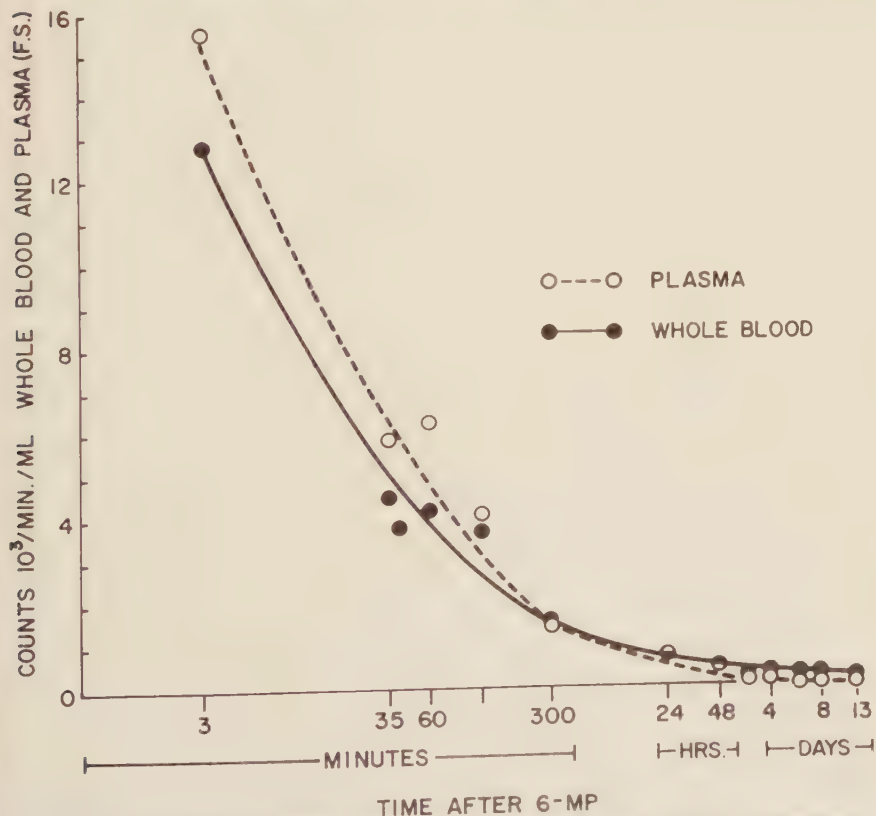


FIGURE 3. Radioactivity in the whole blood and plasma of the adult after the injection of 6-MP-6-S<sup>35</sup>.

greater than that given the adult. The radioactivities observed in the blood of the child were higher than those in the adult by approximately the same proportion.

From chromatographic evidence it appears that 6-MP represents approximately 50 per cent of the activity observed in the plasma initially. On this assumption, from the data in the adult, the half-time of 6-MP in the blood is 90 minutes.

Significant radioactivity (90 counts/min./ml.) was present in the cerebrospinal fluid of the child five minutes after the injection of 6-MP, and it reached a level of 900 counts/min./ml. at 30 minutes. At 24 hours, activity was the same as at five minutes, and at nine days no significant activity was detected. At the latter period it will be recalled that there was activity associated with whole blood but not in plasma. The cerebrospinal fluid at 30 minutes contained 66 mgm./100 ml. total protein and had a cell count of 992, of which 98 per cent were mononuclears and 2 per cent polymorphonuclears. These elevated values of total protein and cell count raise the question of whether increased permeability of the blood-brain barrier accounted for the activity found in the spinal fluid.

The spinal-fluid protein of the adult determined on the 60-minute sample was 22 mgm./100 ml., *i. e.*, within normal limits. Nevertheless, significant activity was present in the spinal fluid at 30 and 60 minutes, the counts being 190 and 260/min./ml., respectively.

FIGURE 4 shows the radioactivity in thousands of counts/min./ml. of whole blood and spinal fluid of the adult (the adjacent 30 and 60 minute bars) and of the child (the 30 minute bar). It is apparent from the diagram that in both the adult and the child, activities in the spinal fluid at 30 and 60 minutes have approximately similar relationships to the blood level. Analysis of the spinal fluid shows that 6-MP represents at least one third the observed activity; apparently 6-MP can cross the blood-brain barrier.

The urinary findings indicate rapid, extensive metabolic transformation of 6-MP. FIGURE 5 shows the cumulative total activity in  $1 \times 10^6$  counts/min. excreted in the urine of the adult plotted against time in hours on a logarithmic scale after the injection of 6-MP. At 24 hours, approximately 60 per cent of the administered radioactivity had been recovered in the urine. Excretion of activity was prompt and rapid, and the linearity of excretion during the first five hours is immediately apparent (FIGURE 5). The excretion curve obtained in the child was similar, although the initial collection was not available.

Urine samples were chromatogrammed to determine the nature and distribution of the various metabolites at different times. FIGURE 6 is an example of the chromatograms obtained; the results are from 3-5 hour urine of the child. The chromatogram, run in isopropanol- $(\text{NH}_4)_2\text{SO}_4$ , shows the presence of 6-thiouric acid, Rf 0.25, 6-MP, Rf 0.40, an unknown metabolite, Rf 0.70, and sulfate, Rf 0.90.

The per cent composition of the radioactivity in the urine of the adult, obtained by similar chromatograms, at different hours after 6-MP, is shown in FIGURE 7. In the urine collection at one hour, the per cent composition of

the radioactivity was accordingly 6-MP 39, 6-thiouric acid 36, unknown 13, and a trace of sulfate. At five hours, 6-MP accounted for 28, thiouric acid for 19, the unknown for 21, and sulfate for 21 per cent of the radioactivity. In the 24-48 hour collection, 6-MP and 6-thiouric acid had decreased to 8 per cent, while sulfate had increased to 75 per cent. The changes with time in the percentage composition of the radioactivity in the urine of the child were similar. In the 3-5 hour urine collection, 6-MP accounted for 15 per cent of the activity, 6-thiouric acid for 15 per cent, while 40 per cent appeared as sulfate, and nearly 20 per cent as the unknown. The proportion of 6-MP and 6-thiouric acid decreased thereafter, while the excretion of radioactive sulfate increased. In the 45½-48½ hour urine collection, sulfate represented 92 per cent of the activity; the unknown, 3 per cent; and there was a trace of 6-thiouric acid.

The data from the adult on the distribution of the various metabolites at different times allow calculation of the distribution of 6-MP in the body and of its clearance from the plasma.

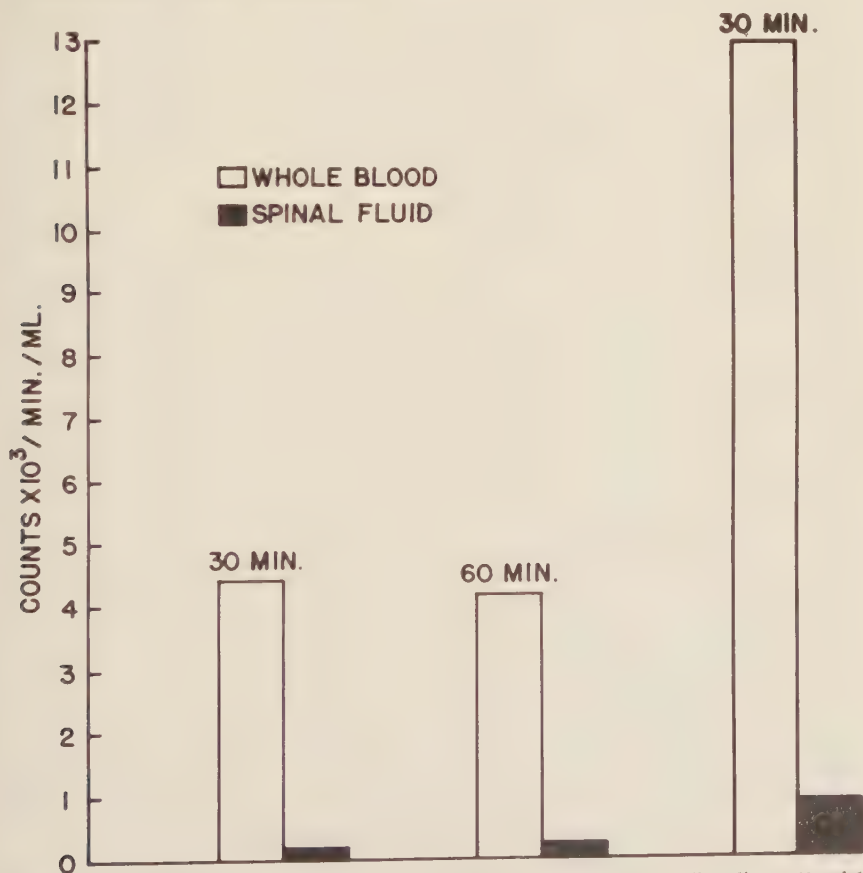


FIGURE 4. Radioactivity in the whole blood and spinal fluid of the adult (two smaller adjacent 30 and 60 minute bars) and of the child (taller 30 minute bar) after the injection of 6-MP-6-S<sup>35</sup>.



TABLE 1 outlines the calculation of the "distribution" of 6-MP in the adult at one hour. This calculation is based on the assumption that the proportion of 6-MP in the total radioactivity remaining in the body is the same as that found in the plasma at this time, *i. e.*, approximately 50 per cent. The calculated figure of 45 liters, in which the total remaining activity is dissolved at one hour, corresponds to a total body-water value of 60 per cent in a 75 kg. man. A similar calculation, carried out with the data at two hours, gave a figure of 56 liters, which corresponds to a total body-water value of 70 per cent. These approximations make it unlikely that 6-MP is confined to the extracellular fluid, but suggest that it is probably distributed generally throughout the total body water at this period.

TABLE 2 summarizes the calculations of the renal clearance of 6-MP in the adult. The total activities in  $1 \times 10^6$  counts/min., which represent 6-MP in the individual urine collections, were calculated from the relative distribution data of the various metabolites (FIGURE 7) and the total activity excreted in each urine collection (FIGURE 5).  $C_{6\text{-MP}}$  was calculated by dividing the activity of 6-MP in the urine by " $P$  mid"  $\times 60$ . A determination of urea clearance, UV/B, (the maximum clearance)<sup>3</sup> in this patient one week before the experiment gave a value of 89 per cent of normal and the clearance, UV/B, taken between hours 2 and 3, gave a value of 103 per cent of normal. It appears reasonable to conclude that this patient's glomerular filtration rate was within normal limits during the experimental period. Three of the five clearance figures

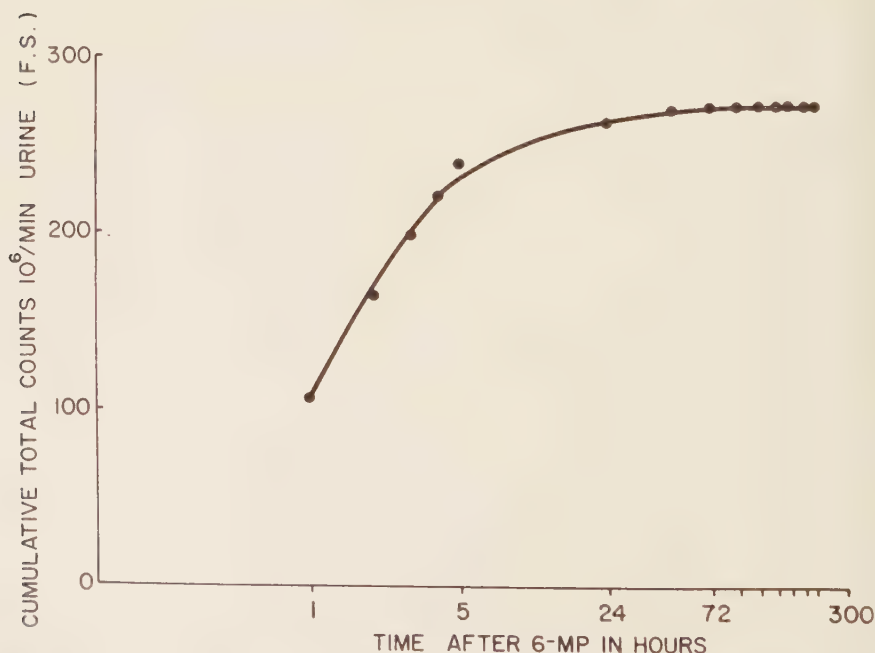


FIGURE 5. Cumulative total radioactivity excreted in the urine of the adult after the injection of 6-MP- $6\text{-S}^{35}$ .

obtained for 6-MP would correspond approximately to the estimated rate of glomerular filtration.<sup>4</sup> The clearance data thus suggest that there is little tubular excretion or reabsorption of 6-MP, and that its excretion in the urine is largely determined by the rate of glomerular filtration. The calculations on both the distribution and clearance of 6-MP are only approximations, based as they are on data with a wide margin of error; the conclusions to be drawn from them must, correspondingly, be considered to be tentative. Neverthe-

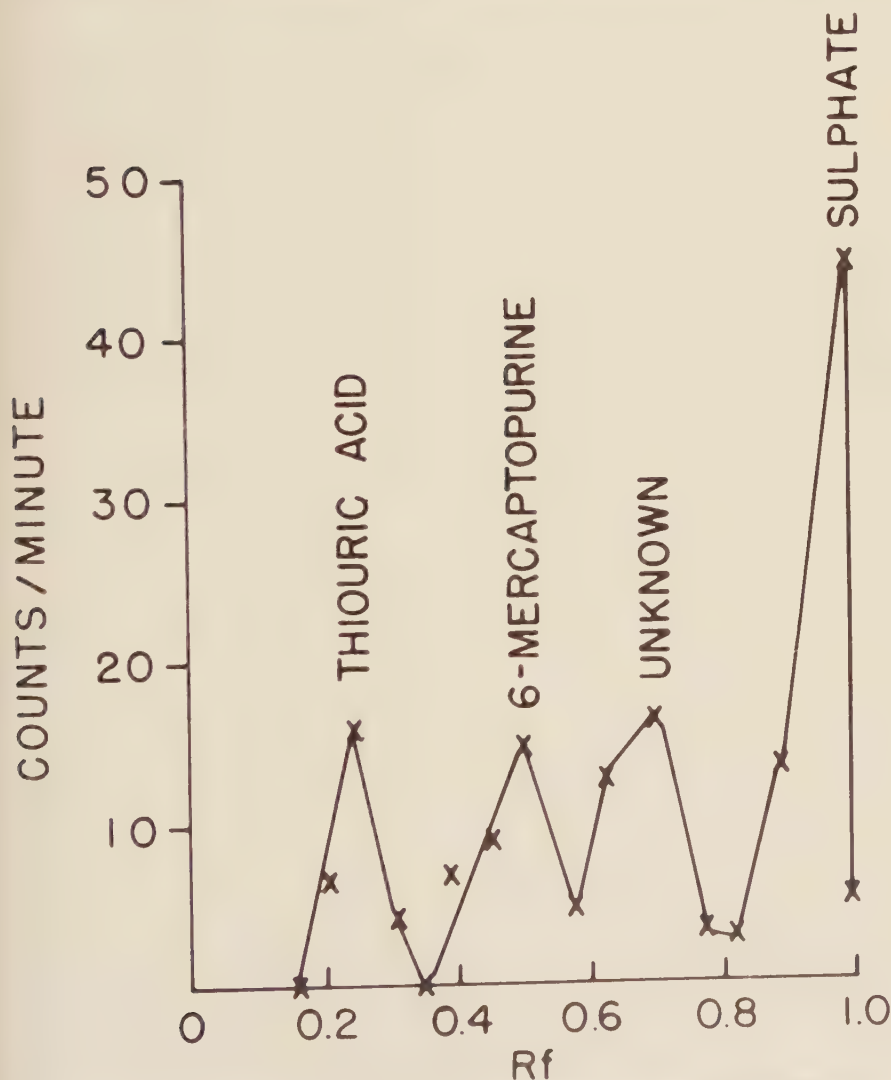


FIGURE 6. Sample chromatogram of the 5-hour urine of the child after the injection of 6-MP-6-S<sup>35</sup>, showing the radioactivity and Rf of various metabolites; chromatogram run in isopropanol-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

less, the results of the calculations do appear reasonable, which supports the conclusions as to distribution and clearance just drawn.

Excretion of radioactivity in the feces of the adult was negligible in both the 24- and 48-hour collections. Less than 1.5 per cent of administered activity appeared in each of the two 24-hour collections.

The radioactivity excreted in each 24-hour urine collection showed a gradual and regular decline until days 16 and 17, at which time the patient received two transfusions, each of 500 ml. of whole blood, and was begun on unlabeled 6-MP. Thereafter, simultaneous with a rapid and extensive decline in the white-cell count and some decrease in the size of the liver, total activities in the 24-hour urine collections continued to decline but in an irregular fashion. Significant radioactivity was still present in the 45-day collection.

*Discussion.* It is convenient for the purpose of discussion to regard the radioactivities, except where indicated otherwise, as 6-MP or as a close derivative of 6-MP. But until the actual metabolites represented in the total radioactivities are identified, it should be borne in mind that different metabolites may predominate in different situations. With this reservation, there was no noticeable difference in the distribution and metabolism of 6-MP-6-S<sup>35</sup> in a child or an adult. Similarly, the presence of acute stem-cell leukemia in the child and of a different type of leukemia—chronic myeloid—in the adult also has not induced any detected difference. The interpretation of the lack of

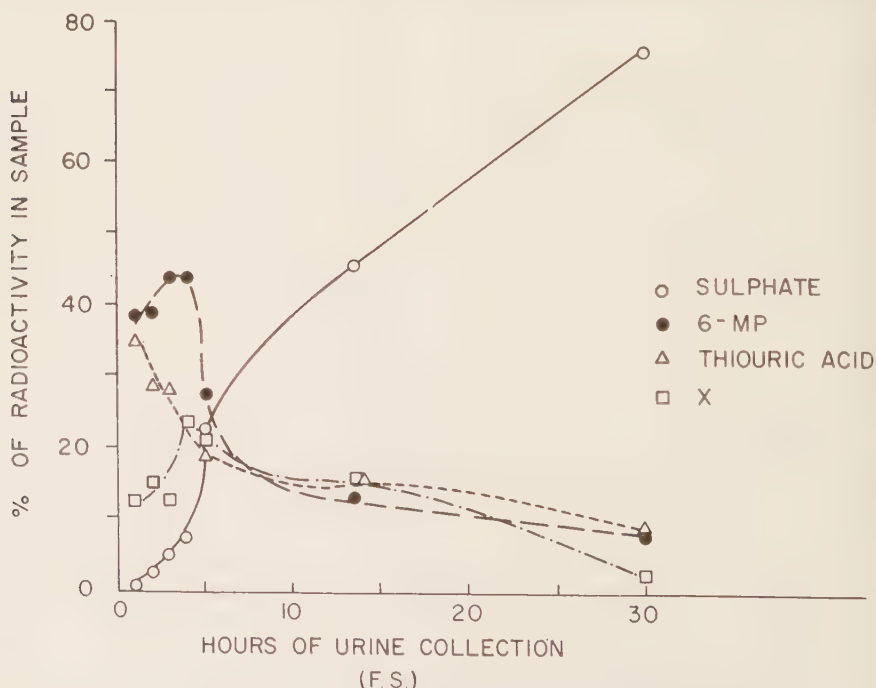


FIGURE 7. The distribution of the various metabolites in the urine of the adult at different times after the injection of 6-MP-6-S<sup>35</sup>, shown as the per cent composition of the radioactivity in the urine.

TABLE 1

CALCULATED "DISTRIBUTION" OF 6-MERCAPTOPURINE IN THE ADULT AT 1 HOUR

- (1)  $391 \times 10^6$  counts/min. were given at 0 time.
- (2)  $106 \times 10^6$  counts/min. appeared in urine 0-60 minutes.
- (3)  $\therefore 285 \times 10^6$  counts min. remained in the body at 60 minutes.
- (4) of this, 50 per cent is assumed to be 6-MP *i.e.*  $143 \times 10^6$  counts/min. in body represented 6 MP.
- (5) at 60 minutes, activity of 6-MP present in plasma was 3150 counts/min./ml.
- (6)  $\therefore$  6-MP was dissolved in  $\frac{143 \times 10^6}{3150} \times \frac{1}{1000}$  liters = 45 liters approximately.

TABLE 2

CLEARANCE OF 6-MERCAPTOPURINE, C<sub>6-MP</sub>, FROM THE BLOOD OF THE ADULT

Time minutes	6-MP in urine cpm* $\times 10^6$	P mid cpm*/ml.	C <sub>6-MP</sub> ml./min.
0-60	41.2	4100	170
60-120	23.4	2500	156
120-180	14.3	1800	132
180-240	10.0	1250	133
240-300	4.8	900	89

\* Radioactivity in counts/minute.

(1) Time in minutes of urine collections after 6-MP.

(2) Total activity which represents 6-MP in urine.

(3) "P mid" represents the calculated activity of 6-MP in the plasma at the mid-point of each time period of urinary collection.

(4) C<sub>6-MP</sub> is the volume of plasma in ml./min. cleared of 6-MP.

difference between the two types of leukemia is complicated, because the adult's disease had been present for at least two years and he died five months after the experiment. It is thus possible that the chronic myeloid leukemia of the adult male might have been becoming acute at the time of the experiment, although the hematological picture, including absence of thrombocytopenia, would not favor this point of view. It is of interest that the treatment received by the child before the experiment—unlabeled 6-MP 2.5 mgm./kg./day for some 130 days and O-diazoacetyl-L-serine 2 mgm./kg./day for approximately 90 days—did not appear to affect the metabolism of 6-MP.

The radioactivities found in the spinal fluid of the child and the adult indicate that 6-MP at a dosage of 6 mgm./kg., given intravenously, passes the blood-brain barrier. The results do not establish that this happens when a dose of 2.5 mgm. kg. or 5 mgm./kg. is given orally. Studies now in progress on the oral administration of 6-MP-6-S<sup>35</sup> show significant radioactivity in the spinal fluid at four hours after 6 mgm./kg. by the oral route—6-MP appears to penetrate the blood-brain barrier.

The urinary findings in both the child and the adult indicate the rapid and extensive transformations of 6-MP. The distribution of the various metabolites in the urine at different times contrasts with the findings in mice,<sup>5</sup> in which sulfate did not appear to the extent found in man. The suggestion that 6-MP is excreted by the kidneys mainly as a result of glomerular filtration might make it possible to calculate a dosage schedule which would maintain a therapeutically desirable blood level of the agent. Further studies are projected with this objective.



Pertinent to the irregularities in the decline in activities found in the urine of the adult, associated with transfusions, unlabeled 6-MP, and a fall in white count, is the finding of activity in both nucleic acids of the tissues of mice after the administration of labeled 6-MP.<sup>5</sup> Preliminary data likewise suggest that there was significant radioactivity associated with highly purified deoxyribose nucleic acid isolated from the leukemic leucocytes of the adult on days 2, 4, and 6 after 6-MP-6-S<sup>35</sup>. The possibility that this represents incorporation in human leucocytes is of interest, in view of the pronounced differences in patterns of purine utilization for nucleic acid synthesis among different species,<sup>6</sup> and the ability of human leukemic leucocytes to utilize adenine, of which 6-MP is an analog, for the synthesis of both ribose nucleic acid and deoxyribose nucleic acid adenine and guanine.<sup>7, 8</sup> Further studies are in progress to determine, if possible, the nature and position of the radioactivity associated with nucleic acids following the administration of 6-MP.

It is obvious that one mechanism by which 6-MP might inhibit growth is by getting itself incorporated into nucleic acids; such a mechanism has been postulated for several purine and pyrimidine analogs.<sup>9, 10</sup> It is also possible that at least some portion of the action of 6-MP might result from its incorporation into nonnucleic acid nucleotides, as in the nucleotide portion of vitamin B<sub>12</sub><sup>11</sup> and in the adenylic coenzymes. The demonstration of incorporation of an abnormal purine into nucleic acid or nucleotide is not proof that this is the sole or even the main mechanism for growth inhibition, but such results should at least add to our knowledge of the structure of these substances.

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# ON THE MECHANISM OF ACTION OF 6-MERCAPTOPURINE

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Elion *et al.*<sup>1</sup> put forward the suggestion that a hypoxanthine-containing metabolite may be an intermediate in the conversion of adenine to guanine in *L. casei*, and that its transformation to a guanine-containing substance might be viewed as a possible site of action of 6-mercaptopurine (6-MP). These authors observed that a 6-mercaptopurine-resistant strain of *L. casei* (MPR), unlike the wild strain, was incapable of using hypoxanthine for growth and grew poorly on adenine in a folic acid-free medium containing thymine, suggesting the scheme presented as FIGURE 1.

When the wild and MPR strains were grown in the presence of 8-C<sup>14</sup>-adenine, the MPR strain accumulated large amounts of inosine, appreciable amounts

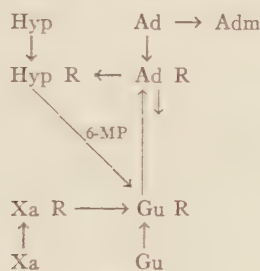


Figure 1

of hypoxanthine, and a small amount of an adenine nucleotide, but no free adenine. These compounds occurred to a much smaller extent in the medium in which the wild strain had been grown.<sup>2</sup> This observation provided further evidence that the MPR strain was resistant to the action of the antagonist because it did not utilize a pathway on which hypoxanthine ribotide is an intermediate.

It has been observed that hypoxanthine is active in preventing the anti-leukemic action of A-methopterin, which is an inhibitor of "de novo" purine synthesis.<sup>3</sup> Results obtained with L1210 leukemia are summarized in TABLE 1.

The data presented in TABLE 1 suggested that hypoxanthine might be utilized as a purine source by the leukemic cells employed.

Studies were then carried out to determine if 6-MP inhibited "de novo" nucleic acid synthesis (as measured by formate incorporation) or "preformed" pathways.<sup>4</sup> Mice bearing sarcoma 180 or adenocarcinoma 755 were used in these experiments. Results summarized in TABLE 2 indicate that 6-MP inhibits "de novo" nucleic acid synthesis but does not significantly inhibit adenine incorporation into combined nucleic acids. Also it has been observed that 6-MP inhibits hypoxanthine incorporation into polynucleotides.<sup>12</sup>

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TABLE 1  
PREVENTION OF THE ANTILEUKEMIC ACTIVITY OF A-METHOPTERIN BY HYPOXANTHINE

Expt. No.	Treatment	Dosage (mgm./kg.)	Life span	
			Days	% Above controls
1	Controls	—	9.2	—
	A-Methopterin	3.0	25.3	175
	A-Methopterin + hypoxanthine	3.0 + 500	8.7	-5
2	Controls	—	9.2	—
	A-Methopterin	3.0	21.4	132
	A-Methopterin + hypoxanthine	3.0 + 500	12.6	37
3	Controls	—	9.4	—
	A-Methopterin	3.0	25.0	165
	A-Methopterin + hypoxanthine	3.0 + 500	10.8	15
	A-Methopterin + hypoxanthine	3.0 + 250	15.9	69
	A-Methopterin + uric acid	3.0 + 500	22.2	136

Note. All therapy initiated at 24 hours and continued every other day for a total of ten injections or until death.

These data suggest that one of the sites of action of 6-MP might be somewhere along the “*de novo*” pathway to nucleotides.

The results obtained in pigeon-liver systems and bacteria have suggested the following metabolic map for hypoxanthine-ribotide synthesis (5, 6, 7, 8, 9 and others):

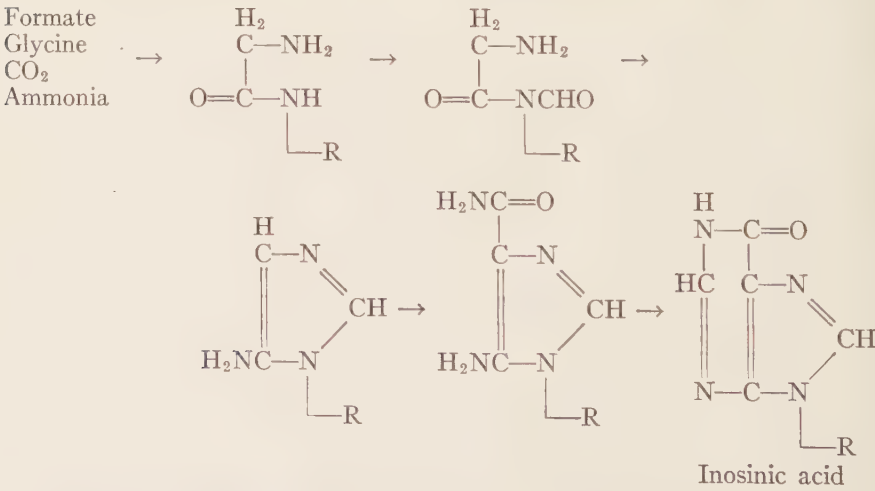


TABLE 2  
 EFFECTS OF 6-MERCAPTOPURINE ON NUCLEIC ACID SYNTHESIS

Labeled compound	Inhibitor	Tumor	Inhibition of incorporation into CNA	
			Tumor	Intestine
Formate	A-Methopterin	Sa 180	+	+
Formate	A-Methopterin	755	+	+
Formate	6-MP	Sa 180	+	+
Formate	6-MP	755	+	+
Adenine	6-MP	Sa 180	—	—
Adenine	6-MP	755	—	—
Hypoxanthine	6-MP	Normal mice		+

Note. + indicates significant inhibition in two experiments with concurrently run controls.

source in bacteria; (2) inosinic acid lies on the "*de novo*" pathway to nucleotide synthesis in the pigeon liver system; (3) 6-mercaptopurine inhibits "*de novo*" nucleic acid synthesis in the mouse; and (4) hypoxanthine prevents the anti-leukemic activity of A-Methopterin in mice.

In cooperation with Doctor Helene Toolan and Doctor C. P. Rhoads of Sloan-Kettering Institute, studies are under way with regard to the metabolism of human tumors growing in the hamster pouch. In the course of these studies, hypoxanthine-8- $C^{14}$  with high specific activity (5.1 mc./mM)\* was injected into hamsters bearing human sarcoma and, after six hours, the DNA and RNA purines from the human tumor, hamster intestine, and hamster liver were isolated and assayed for radioactivity. The results of these experiments and similar experiments in which  $C^{14}$ -formate and  $C^{14}$ -xanthine were employed are summarized in TABLE 3.

These results suggest that hypoxanthine can act as a precursor of polynucleotide adenine and guanine in certain hamster tissues and in human sarcoma. Repeat experiments with labeled formate and hypoxanthine have confirmed the results presented in TABLE 3.

Earlier work by Brown and associate<sup>10</sup> which failed to demonstrate incorporation of  $N^{14}$ -hypoxanthine into the polynucleotides of the rat might have been the result of species variation or of a high inosinic acid pool accompanied by lesser sensitivity of mass-spectrometer methods. It has been shown that inosine can act as a precursor of polynucleotide purines in *L. casei*.<sup>11</sup>

In any event, these preliminary results suggest the possibility that inosinic acid may be an intermediate on the "*de novo*" pathway to polynucleotide adenine and guanine in hamster and human tissue. Similar 6-hour experiments with  $C^{14}$  adenine and  $C^{14}$  guanine show that these purines are not equal precursors of adenine and guanine, although adenine is slowly converted to polynucleotide guanine.

For purposes of comparison, the above results are presented along with other data on incorporation of labeled compounds into polynucleotide purines of the mouse and rat. In TABLE 4 these data are presented in terms of per cent of

\* Prepared by Doctor John Montgomery, Southern Research Institute.



TABLE 3

INCORPORATION OF HYPOXANTHINE, XANTHINE, AND FORMATE INTO NUCLEIC ACID PURINES

Compound administered	Human tumor				Intestine				Liver			
	DNA		RNA		DNA		RNA		DNA		RNA	
	Ad	Gu	Ad	Gu	Ad	Gu	Ad	Gu	Ad	Gu	Ad	Gu
Hypoxanthine...			0.2	0.14	0.4	0.3	0.7	0.5			0.3	0.3
Hypoxanthine ..			<0.2	0.17	1.2	0.9	2.2	1.6			0.4	0.5
Xanthine.....			<0.02	<0.02	<0.02	<0.02	<0.02	<0.02			<0.02	<0.02
Formate.....	0.4	0.5	1.0	0.9	1.2	1.0	1.9	1.6	0.1	0.1	0.1	0.1

Note. Values in cps/ $\mu$ . 30  $\mu$ c per 75 gm. injected in all experiments. Specific activity of hypoxanthine 5.1 mc./mM; xanthine 3.7 mc./mM; formate 3.6 mc./mM.

adenine and guanine derived from the labeled precursors; however, because of differences in experimental techniques, they can be considered only as qualitative comparisons.

If one accepts the previous interpretation of these results then the "*de novo*" pathway to nucleic acid synthesis could be represented as shown in FIGURE 2.

Utilizing this concept for purposes of discussion it is possible to rationalize certain experimental results as shown in FIGURE 3.

It is certainly apparent that the map presented in FIGURE 3 is no more than a current attempt to fit together data (obtained in many biologic systems) into a temporary working hypothesis. It is probable that many of these "guesses" will prove to be untenable, but one of the purposes of this conference is to exchange results, ideas, and concepts. Our working hypothesis will undoubtedly have changed by the end of this conference.

The observations against which the map presented in FIGURE 3 has been tested are listed below:

(1) Formate and glycine are incorporated into polynucleotide adenine and guanine at approximately the same rate. 4-Amino-5-imidazole carboxamide

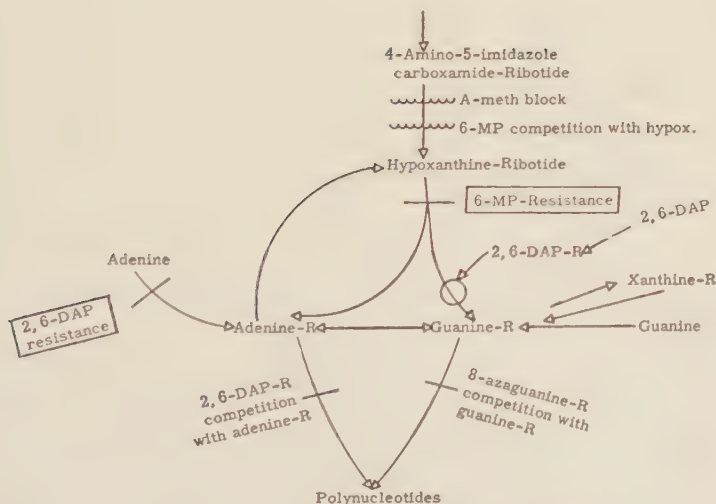
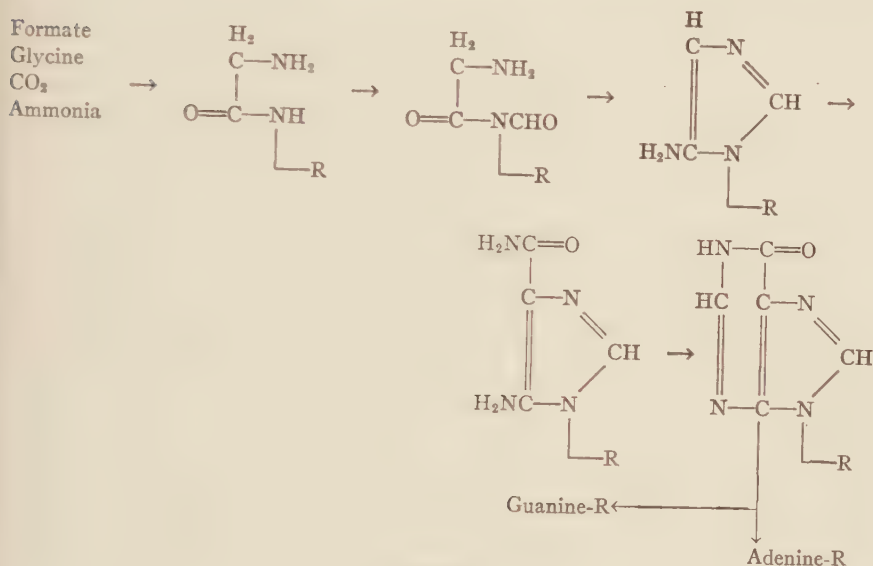
TABLE 4

ESTIMATES ON THE PERCENTAGE OF POLYNUCLEOTIDE PURINES DERIVED FROM LABELED COMPOUNDS

Labeled compound	Animal	Tissue	Approx. % RNA purines derived from labeled compound		Comments	Ref.
			Adenine	Guanine		
Formate	Hamster	Intestine	0.13	0.15	1 inj; 6 hours	12
4-Amino-5-imidazole carboxamide	Mouse	Liver	2.05	1.53	Feeding 6 days; 24 hours	13
Hypoxanthine	Hamster	Intestine	0.2	0.18	1 inj; 6 hours	12
Adenine	Rat	Intestine	2.6	0.7	1 inj; 24 hours	14
Xanthine	Hamster	Intestine	<0.002	<0.002	1 inj; 6 hours	12

Note. These are rough approximations calculated on data obtained on different species, with differing routes of administration and experimental periods. Under comments, the reference to time has to do with the period after last administration at which animals were sacrificed.

and hypoxanthine are likewise incorporated into both adenine and guanine at the same rate. Adenine goes into polynucleotide adenine rapidly and slowly into guanine. Guanine is incorporated into polynucleotide guanine. 2,6-Diaminopurine is converted to polynucleotide guanine in considerable amounts. (2) A-Methopterin and 6-MP inhibit "de novo" nucleic acid synthesis, but



(Note: this is an attempt to fit together both animal and bacterial data. Some pathways used by bacteria do not appear to be operative in animals.)

fail to inhibit adenine incorporation. 6-MP appears to inhibit hypoxanthine incorporation into polynucleotides.

(3) Hypoxanthine, xanthine, guanine, or adenine will reverse 6-MP in *L. casei*.

(4) 6-MP-resistant *L. casei* cannot utilize hypoxanthine and build up labeled inosinic acid from adenine-C<sup>14</sup>.

(5) Hypoxanthine will prevent the antileukemic activity of A-Methopterin (L1210 leukemia).

(6) 6-MP-resistant or 8-azaguanine-dependent L1210 leukemia is more susceptible to A-Methopterin than the parent L1210 leukemia.

(7) 2,6-Diaminopurine and 8-azaguanine are incorporated poorly into polynucleotides of 8-azaguanine-dependent leukemia L1210.

(8) A-Methopterin + 6-MP or azaserine + 6-MP are synergistic with regard to inhibition of L1210 leukemia.

(9) 6-MP-resistant *L. casei* grows well on xanthine, guanine; poorly on adenine or adenylic acid *b*, and scarcely at all on hypoxanthine. This mutant is sensitive to 2,6-diaminopurine of 8-azaadenine (reversible by adenine).

(10) 2,6-Diaminopurine-resistant *L. casei* are not inhibited by purine or 8-azaadenine (antiadenines) and is more sensitive to 6-MP than the wild strain. This organism has a diminished ability to incorporate adenine or diaminopurine.

(11) 6-MP + purine is synergistic with regard to inhibition of *L. casei*, but the combination of 6-MP + 2,6-diaminopurine is mutually antagonistic.

Some of these observations can be fitted into the current scheme, others cannot be. Working hypotheses such as this one are sometimes useful to their author, but often are confusing and misleading to others; therefore we hasten to tag this one with all of the common terminology which implies unconfirmed, possible, suggestive and, most important, preliminary.

It should also be pointed out that maps such as the one presented cannot depict the actual sites of competition, blocks, and poorly used pathways which may manifest resistance. These sites are, we assume, enzymic. Attempts have been made to point to areas in biochemical events where antagonism, or failure of antagonists, might explain certain available experimental results.

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# THE NATURAL HISTORY OF UNTREATED ACUTE LEUKEMIA\*

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Untreated acute leukemia is a complex, fatal, neoplastic disease characterized by protean clinical manifestations, a wide diversity of sometimes confusing laboratory findings, and an exceedingly variable time course. Not infrequently the diagnosis is difficult, the management discouraging, and the evaluation of results misleading. While the clinical picture and course of the disease may vary greatly from one patient to the next, the over-all pattern of a group of patients is quite characteristic. The purpose of this review is to examine, in a quantitative fashion, the pattern of untreated acute leukemia, with particular reference to those characteristics which may form a basis for the evaluation of therapeutic agents.

While leukemia as a recognized disease is over 100 years old,<sup>1, 2, 3</sup> the recorded history of effective palliative treatment of acute leukemia dates from the introduction of Aminopterin by Farber and associates<sup>4</sup> in 1948. Since then clinical trials of a number of chemotherapeutic agents have been reported. As some of these compounds have shown an apparent differential success in the treatment of the various morphologic types of leukemia, and even differences in the effectiveness of treatment of the same disease in the adult and the child, it will be necessary to consider the characteristic pattern of each disease in appropriate detail.

The ideal basis for evaluation of the natural history of leukemia would be the carefully compiled results of a series of patients selected at random who were carefully and objectively followed from the onset of disease to death. A random series implies that every patient with acute leukemia has an equal and independent opportunity to be included in the study. As far as can be determined, no such series exists.‡ It is accordingly essential to examine the limitations of the data from which this review is assembled.

Certain sources of bias and deficiencies of reporting severely limit the information from which a complete picture of acute leukemia can be compiled.

- (1) Diagnosis is frequently incomplete. Criteria<sup>6</sup> exist for the morphological differentiation of the several types of leukemia, but the application of these criteria is extremely difficult in the more acute forms, hence many authors report data simply as "acute leukemia."
- (2) Data may be combined for experiences on adults and children. No unassailable prior proof is known to exist that the prognosis is identical.
- (3) Reporting of cases is incomplete in some articles. All cases should be reported, even those moribund upon admission, for most complete evaluation of results.

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‡ The facilities available to Farber and associates<sup>5</sup> suggest that their treated series (est. > 90 per cent of all children afflicted with leukemia in New England), when published, may approach a random series.

- (4) The nature of present day specialized hospital practice makes it difficult to assemble a series of patients representative of the age and sex distribution characteristic of acute leukemia; e.g., children only are seen in some hospitals.
- (5) An excellent reputation of the investigator and/or his hospital facilities may attract an undue proportion of cases which are difficult both in diagnosis and treatment.
- (6) The average survival times frequently reported are not necessarily representative of the typical patient afflicted with leukemia and, therefore, cannot be utilized reliably.
- (7) Observers, being human, have a tendency to observe and report matters of current interest. For example, the literature of the past three years discloses case reports of about 30 spontaneous remissions in leukemia. This figure seems inordinately large in comparison with the total of 49 reported up to 1951 by Southam and associates.<sup>7</sup> Further, the present practice of hospitalization of children for study provides a much better opportunity to observe the detailed course of the disease than outpatient care can provide.

These and other factors combine to introduce a large element of uncertainty into the data summarized in the sections which follow. The above difficulties are listed in condolence, not criticism. It seems regrettable that such an enormous number of clinical research hours must be lost because of inadequate reporting and analysis of information which may have been available at the time of writing.

An effort has been made to review applicable material from available publications which might provide a reasonable picture of the characteristic course of the patient seen in a research hospital. No claim can be made that this review represents the picture for all patients having this disease. The base line figures on untreated leukemia derived from this study should be accepted and used only with this appropriate reservation. If this paper stimulates the critical analysis of truly representative unreported data on untreated leukemia, and if such analysis proves its conclusions faulty, then a most useful purpose will have been accomplished.

A review of the clinical manifestations of acute leukemia is certainly unnecessary for the participants of this meeting. Such a survey has been most ably accomplished by a number of authors including Forkner,<sup>8</sup> Sturgis,<sup>9</sup> Southam and associates,<sup>7</sup> and Windeyer and Stewart.<sup>10</sup> Our attention will be focused upon only those features which are subject to some form of reliable quantitative measurement of possible merit in the evaluation of new methods of therapy. This statement is not made to decry the value of the "clinical impression." The merit of this method of evaluation is in direct proportion to the clinical experience of the investigator, thus leaving the younger man at a distinct disadvantage.

*Scope of the problem.* Leukemia is a major health hazard. In the United States, Sacks and Seeman<sup>11</sup> have estimated that more than 5000 persons per year have died from this disease since 1940. Gilliam's<sup>12</sup> analysis of death

certificates (United States) for 1949 indicated 8102 deaths attributed to all forms of leukemia. In view of the probability of a number of unreported cases of leukemia, especially of the aleukemic type, it is likely that these represent conservative estimates. The recorded death rate for leukemia in the United States is rising<sup>11, 12</sup> and similar rising rates are quoted from Scotland<sup>13</sup> and from Denmark.<sup>14</sup> Some of this increase may be attributed to improvements in diagnostic facilities, but the continued rise in cities having a long record of good medical facilities and an unchanging proportion of aleukemic leukemias<sup>12</sup> would suggest that some of this increase is not statistical artifact.

*Incidence and prevalence of leukemia.* Gilliam<sup>12</sup> has cogently emphasized that an analysis of deaths in leukemia cannot provide an estimate of the incidence of leukemia. Incidence is a measure of the risk of dying of leukemia for a specified population, for example, for all persons in the 0-5-year age group taken over a specified interval of time, for example, one year. As an example, from Gilliam's data there were 235 deaths from lymphocytic leukemia in white males in the first five years of life, and 218 reported for the 60th to 65th year of life. The risk of dying of leukemia in the first five years is 33 per million per year but, because of the much smaller group of persons alive between the ages of 60 and 65, the risk of dying of lymphocytic leukemia in this time interval is 79 per million per year, a little more than twice that of infancy.

The prevalence of leukemia, that is, the number of patients alive who are afflicted with the disease at any one time, depends both upon the number of cases diagnosed and the survival time of these patients. It is essential to arrive first at some estimate of the relative numbers of patients having acute and chronic leukemias. Sacks and Seeman<sup>11</sup> analyzed the 97 death certificates on which the chronicity of leukemia was indicated out of a total of 154 certificates from the city of Baltimore. Sixty-one per cent of these recorded deaths were listed as acute leukemia.

There are three large series<sup>13, 15, 16</sup> of patients in which the relative numbers of acute\* and chronic patients are recorded. These are summarized in TABLE 1. The differences in the ratios of leukemia by cell type and acute or chronic classification are too great to be attributed to chance alone. Granted that the differentiation of acute leukemias by cell type is difficult, the differences between the relative numbers of acute and chronic leukemias, regardless of cell type, given in this table is still greater than can be expected on the basis of chance alone.

One must conclude, therefore, that either the institutions represented by these three series serve different types of populations, or that the diagnostic criteria differ among the three investigators. The report of Gauld, Innes, and Robson<sup>13</sup> is believed to include a "very large proportion of the total number of cases of leukemia occurring in the eastern half of Scotland during the 14 years under review." It is believed that there are no serious diagnostic differences between the criteria of at least one of these investigators and those specified by the Committee for Clarification of the Nomenclature of Diseases of the

\* In this analysis, all monocytic leukemias were considered "acute" and the fraction of each cell type given by the total of the three authors was taken as the expected frequency for a  $\chi^2$  analysis.  $P(\chi^2) < 0.001$ .

TABLE 1

DISTRIBUTION OF LEUKEMIA IN BOTH ADULTS AND CHILDREN BY CELL TYPE AND CHRONICITY

Authors	Date	Number of Cases						Total
		Lymphocytic		Granulocytic		Mono- cytic* total	% Acute Leu- kemia	
		Acute	Chronic	Acute	Chronic			
Rosenthal and Harris <sup>15</sup> . . . . .	1935	32	105	137	159	9	39%	442
Bethe <sup>16</sup> . . . . .	1943	112	104	44	131	104	51%	495
Gauld, Innes, and Robson <sup>13</sup> . . . . .	1952	144	207	70	169	57	41%	647
Data of Gauld, Innes, and Rob- son as per cent of all leukemias as per cent of all acute leu- kemias . . . . .		22	32	11	26	9		100
		53		26		21		100

\* All monocytic leukemia is considered "acute."

Blood and Blood-forming Organs.<sup>5</sup> On the basis of this agreement and the inclusive nature of the survey, this report is likely to provide the present best estimate of the relative frequency of the various types of leukemia. These authors would indicate that 41 per cent of all leukemia diagnoses made are acute leukemia. This calculation is in reasonable agreement with the experience of Rubnitz,<sup>17</sup> in which, of 100 consecutive cases at Nebraska, 38 were "acute" leukemia.

Applying this estimate<sup>15</sup> to the total deaths from leukemia in the United States,<sup>12</sup> approximately 3300 persons died of acute leukemia, 4800 persons of chronic leukemia, in 1949. The most probable survival of an untreated patient afflicted with acute leukemia is not greatly in excess of 0.25 year; that of a patient having chronic leukemia, approximately 2.5 years. To account for this number of deaths, there should be about 825 patients with acute leukemia and approximately 12,000 patients with chronic leukemia under treatment at all times in the United States. It must be emphasized that *these figures are order-of-magnitude estimates only*, the results of approximate calculations on data of questionable validity.

Data on the fraction of hospital admissions for leukemia vary markedly with the nature of the reporting center.<sup>13, 14, 15, 20</sup> Values may be found in the range of 50 to 500 patients having leukemia per 100,000 admissions.

Since many authors will wish to report combined series of adults and children, it is desirable to estimate the relative numbers of patients having acute leukemia in each of these age groups for the several morphologic cell types. For the purpose of this analysis, a "child" is arbitrarily taken as age 14 or less. In assembling some of the data, it was necessary to interpolate, *e.g.*, age distributions might be given to the nearest five years only. The results are assembled in TABLE 2.

On the basis of the relative numbers of adults and children reported by the various authors<sup>7, 13, 16, 21, 22, 23</sup> for each of the cell types listed, it will be noted that acute leukemia is not necessarily a disease of children, about 44 per cent of the cases from those series reporting both adults and children are included in this age group. In view of the demonstrated differences in morphologic



TABLE 2  
AN ESTIMATE OF THE RELATIVE FREQUENCY OF THE VARIOUS TYPES OF ACUTE LEUKEMIA  
IN ADULTS AND CHILDREN

	Per cent of all acute leukemias*				
	Unclassified	Lymphocytic	Granulocytic	Monocytic	Total
Children.....	4	31	7	2	44
Adults.....	2	16	21	17	56

\* Combined data of references 7, 13, 16, 21, 22, 23.

classification of leukemia, this combination of data in TABLE 2 must be considered an approximation.

In childhood, lymphocytic leukemia is overwhelmingly the most common type, followed by granulocytic and monocytic leukemia in that order. The relative frequency of cell types shown is in substantial agreement with an earlier survey of the pediatric literature<sup>24</sup> based upon a different group of authors.

In adults, acute leukemias appear approximately divided equally among the three cell types. In scanning a large number of articles for this review, many of the small "treatment" series for acute leukemia show an apparent deficiency of cases of monocytic leukemia in children, and of lymphocytic leukemia in adults. The proportions of "unclassified leukemias" shown for both the adult-child categories (TABLE 2) reflect the difficulty of morphologic diagnosis of acute leukemia. The uncertainty of the data as shown is possibly as great or greater than the percentage of "unclassified" leukemias.

*Age distribution in the leukemias.* In FIGURE 1 is illustrated the relative age distribution of the principal types of leukemias. Note that acute lymphocytic leukemia is principally a disease of children, but may be found at any age. This apportionment is a markedly skewed distribution of age and is approximated by a log-normal distribution. For reasons which will be more evident later, an average age is not a suitable measure of this group, but a median age (that of the middle patient of the series, 8.3 years) is more appropriate. The age distribution of acute granulocytic leukemia extends from the newborn to senescence, with equal numbers of patients younger and older than 29 years. The age distribution of monocytic leukemia is in general similar, but the peak value is at 41 years. Chronic granulocytic leukemia is a disease of all age groups from infancy to extreme age, but is concentrated in the middle years with a peak at age 50.<sup>13</sup> Chronic lymphocytic leukemia\* is almost exclusively a disease of adults, primarily those of the geriatric age group, the median age being approximately 65 years.<sup>13</sup>

TABLE 3 duplicates FIGURE 1 in tabular form for convenience of comparisons with other series. In childhood leukemias, the median age for each type of

\* No well authenticated case reports of this disease in children were noted, but an unusual case might be anticipated from the age distribution. Chronic monocytic leukemia does exist, but its frequency is so small (ca. 2 per cent of monocytic leukemias) that for purposes of calculation all monocytic leukemias can be conveniently considered as a single disease. It should be noted that chronic granulocytic leukemia does occur in children, but probably does not comprise over 2 to 5 per cent of all childhood leukemias.<sup>24, 25</sup> This subject is adequately treated by Cooke.<sup>26</sup>

leukemia (and the 95 per cent confidence limits of this median) is: lymphocytic, 3.7 years (3.4-4.1); granulocytic, 5.5 years (4.6-6.5); and monocytic, 6.5 years (4.5-8.0).

These data are not age-corrected incidence figures in a true sense of the word, *i.e.*, they are not corrected in proportion to the fractions of all persons born who remain alive to have the disease in the older age groups. For this reason, as the treatment of other diseases improves, the age distribution of each of these types of leukemia may be expected to shift upward, since increasing numbers of persons will live to be "at risk."

*Sex preponderance.* A summary of sex preponderance assembled from the articles reviewed is presented in TABLE 4. In each instance in which an adequate number of cases (>50) was available, the confidence limits of the sex ratio for males exceeded 50 per cent. We may conclude that acute leukemia is predominantly a disease of males, whether in children or adults.

*Notes on the etiology and nature of leukemia.* The understanding of the

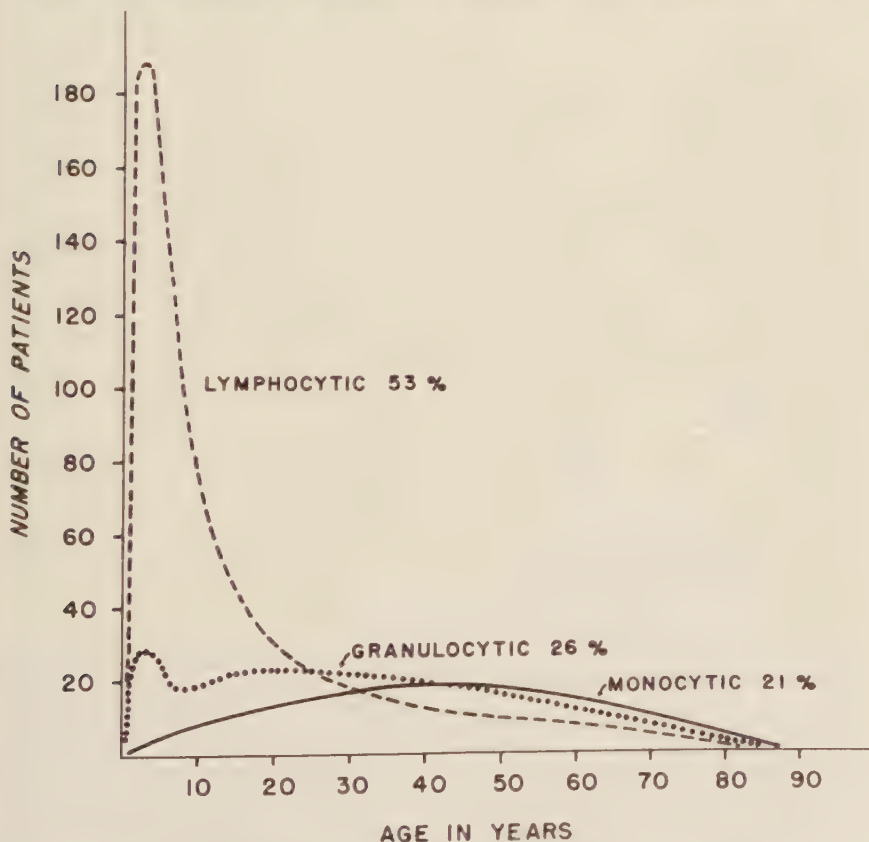


FIGURE 1. Estimated relative numbers and ages of 1000 patients having acute leukemia. The "hump" in the curve for granulocytic leukemia of childhood is probably an artifact arising from failure to distinguish granulocytic and lymphocytic leukemia in this age group. See footnote to TABLE 3 for method of derivation of this figure.

TABLE 3

AN ESTIMATED DISTRIBUTION FOR 1000 CASES OF ACUTE LEUKEMIA DERIVED FROM SEVERAL SOURCES

Age	Lymphocytic	Granulocytic	Monocytic	Total
0-5	189	29	6	224
10	105	17	7	129
15	61	22	9	92
20	33	24	12	69
25	25	17	14	56
30	22	22	16	60
35	17	18	17	52
40	14	18	19	51
45	12	18	18	48
50	10	16	17	43
55	10	14	17	41
60	9	12	16	37
65	8	11	15	34
70	6	9	11	26
75	5	7	9	21
80	3	3	4	10
85	1	2	2	5
Over 90	—	1	1	2
Total	530	260	210	1000
Median age	8.3	29	43	

course of this disease and its rational treatment would be much simpler with a more complete knowledge of the etiology.

Ionizing radiation is now established as a contributing factor in increasing the incidence of leukemia.<sup>46, 47</sup> The incidence of leukemia in radiologists is approximately ten times greater than that of other physicians.<sup>46</sup> This type of radiation is probably, in most instances, a very chronic type of exposure, but single large exposures to radiation may also be a factor. Folley and associates<sup>48</sup> have analyzed the incidence of leukemia in survivors of the atomic bomb explosions in Japan. Within one kilometer of the epicenter of the explosion, the leukemia incidence is approximately fifty times that in the general population, decreasing out to no appreciable increase at more than two kilometers. All ages appear to share the increased incidence of leukemia, but Moloney<sup>49</sup> has pointed out a remarkable absence of chronic lymphocytic leukemia in adult Japanese both in the exposed and control populations. It was stated that adequate numbers of males in the geriatric age groups were examined for evidence of leukemia.

The treatment of polycythemia vera with radioactive phosphorus has been reported by Stroebel<sup>50</sup> to be followed by *acute* leukemia\* in eight patients (3.3 per cent). Two similar instances in 80 patients have been noted in this laboratory. (This disorder should not be confused with the more usual chronic granulocytic leukemia sometimes noted in the terminal stages of this disease.) In addition, Seaman<sup>51</sup> has noted morphologic evidence to suggest a *change of neoplastic cell type* in an acute terminal phase of four patients having chronic leukemia.

\* Stroebel's data indicates that this risk is small in comparison with the risk of vascular complications of the untreated disease, e.g., 26 per cent of his series had some type of vascular accident prior to the use of P.<sup>52</sup>

TABLE 4  
SEX PREPONDERANCE BY AGE GROUP AND TYPE OF LEUKEMIA

	Type of leukemia									
	Unclassified		Lymphocytic		Granulocytic		Monocytic		All	
	M	F	M	F	M	F	M	F	M	F
Children*										
Patients.....	29	21	250	191	63	34	17	12	359	258
Per cent.....	58	42	57	43	65	35	59	41	58	42
95% confidence limits of % M	(44-72)		(52-62)		(56-74)		(41-77)		(54-62)	
Adults†										
Patients.....	10	7	29	9	74	41	107	55	220	112
Per cent.....	59	41	76	24	64	36	66	34	66	34
95% confidence limits of % M	(36-82)		(62-90)		(54-74)		(59-73)		(61-71)	

\* Data combined from references 7, 19, 20, 21, 22, 23, 26 through 41 and, for monocytic leukemia, those of FIGURE 2.

† Data combined from references 7, 19, 22, 23, 26, 29, 31, 38 through 45 and, for monocytic leukemia, those of FIGURE 2.

Leukemia has also been noted to develop in persons exposed to benzol or tars,<sup>52</sup> but any direct relationship is extremely difficult to establish. Bernard and Braier<sup>53</sup> review this problem and present several recent personal cases. A review of the actions of benzol is given by Selling and Osgood.<sup>54</sup>

Johnsson<sup>55</sup> reports a case of acute leukemia following marrow hypoplasia. Bassen and Kohn<sup>56</sup> have reviewed the literature and discussed the relation of agranulocytosis to leukemia of childhood, both as an initial event and as a prelude to spontaneous remission.

*All the contributing agents listed above may have in common a period of hypoplasia followed by a period of relatively increased proliferation of marrow cells and thus possibly an increased chance of a neoplastic mutation during the hyperplastic period.*

Familial leukemia has been reviewed by Decastello<sup>57</sup> and extensively studied by Videbaek.<sup>58</sup> The individual family reports of Thiersch,<sup>59</sup> Anderson,<sup>60</sup> and Portmann and Robinson<sup>61</sup> are also of interest. In several familial instances reported,<sup>58, 60</sup> the cell type of leukemia varied between the patient and his affected relative. "Congenital leukemia" has been described in a number of instances (Söderhjelm and Ranström;<sup>62</sup> Bernhard, Gore, and Kilby<sup>63</sup>) and leukemia in pregnancy has been repeatedly reported (Auer<sup>64</sup> and Er<sup>65</sup>). *As far as can be determined from such reports, the leukemic children have healthy mothers and the leukemic mothers give birth to children having no evidence of leukemia.* Continued observation will be needed to determine the role of heredity in acute leukemia.

The etiology of leukemia might be clarified by a more detailed knowledge of the cytologic and biochemical changes which precede the clinical disease as we commonly recognize it. For example, in chronic granulocytic leukemia, morphologic changes in the marrow have preceded changes in the peripheral blood or clinical manifestations by more than two years.<sup>66</sup> Diagnostic blood changes have preceded clinical signs by a like period.<sup>67, 68</sup> Moloney and Lange<sup>69</sup> have



recently observed that an absolute blood basophilia ( $>200$  cells/mm.<sup>3</sup>) coupled with a low alkaline phosphatase activity of the separated leukocytes preceded by "many months" the morphologic changes in blood or marrow diagnostic of the disease. In the acute leukemias no such criteria were noted.

Block, Jacobson, and Bethard<sup>70</sup> have reported in detail the early clinical and morphologic findings in 12 adults who later developed acute leukemia. In the "preleukemic phase," clinical and hematologic findings were characterized by malaise, noncontributing physical signs and anemia, thrombocytopenia or leukopenia, singly or in combination. Marrow hyperactivity of the erythrocytic series (with anemia) did not appear uncommon. The duration of these non-specific abnormalities varied from 2½ to 20+ months. Examination of the individual case reports indicated that the time interval in which the blood and marrow picture changed from nonspecific abnormality to that of unquestionable acute leukemia may have been one month or less in 5 of the 11 cases in which such an estimate could be made. This interval was less than 1.5 months in the similar case reported by André *et al.*<sup>71</sup>

In such cases as these, without information prior to diagnosis, some of us would feel little hesitancy in placing the clinical onset of the disease during the period in which there were no diagnostic marrow changes! While diagnostic criteria may vary among different centers and so increase these very short (*ca.* one month) "induction times," or marrow sampling errors<sup>72</sup> may add to the uncertainty of these data, these cases pose the question: "What is the primary nature of the change in acute leukemia, morphologic or biochemical, and in which cell series do these changes first occur?"

While association of acute leukemia with infectious disease in children (and possibly comparisons with the virus etiology of fowl leukosis) may have led to considerations of an infectious etiology,<sup>73, 74, 75</sup> no infectious agent in man has been demonstrated. To the numerous unsuccessful attempts to transmit leukemia from man to man reviewed by Windeyer and Stewart,<sup>10</sup> should be added the negative results of Lanman, Bierman, and Byron<sup>76</sup> and of Wallerstein.<sup>77</sup>

Morphologic studies of the peripheral blood and marrow of patients afflicted with leukemia are characterized by cells of abnormal morphology and distribution in numbers. In the acute leukemias there is a predominance of the immature forms, with changes in nuclear structure analogous to some seen in carcinoma. Cultures of these cells in this laboratory demonstrate no reversion toward normal morphology when grown in identical media in which normal marrow cells show continued normal morphology.

The widespread distribution of leukemic cells at necropsy, the occasional formation of localized tumors such as chloroma and the studies of Custer<sup>78</sup> on the interrelationship of leukemia and the other lymphomas, all tend to indicate a close parallelism to recognized neoplastic disease.

At the cellular level, the relationship of the biochemical properties of the leukemic cell to those of malignant tissue is not completely investigated. In chronic granulocytic leukemia, the existence of high levels of histamine is well established by Code and Macdonald,<sup>79</sup> Valentine and Lawrence,<sup>80</sup> Shimkin and associates,<sup>81</sup> Kelemen, Bikich, *et al.*,<sup>82</sup> and Thiersch.<sup>83</sup> Relevant to acute leukemia is the observation of Shimkin *et al.*<sup>81</sup> that the elevated histamine level

of chronic granulocytic leukemia is usually not found in the acute granulocytic leukemia and in those chronic leukemias of short duration. Code and MacDonald<sup>79</sup> and Thiersch<sup>84</sup> also noted low values in lymphocytic and monocytic leukemias. A comprehensive review of the cytochemical aspects of the leukemic cell has been made by Rabinovitch<sup>85</sup> and by Dempsey and Wislocki,<sup>86</sup> who were unable to find a characteristic pattern for these cells.

Valentine's review<sup>87</sup> of the biochemical studies of leukocytes in man summarizes the current status of quantitative determinations in contrast to the more qualitative cytochemical investigations. Manometric studies on the intact cell indicate no characteristic abnormality of respiration or glycolysis of the leukemic cell. Studies on the glycogen content of leukocytes indicate that manufacture and storage of glycogen is essentially limited to the granulocytic series of cells at the myelocyte or more mature stage. Studies indicate that sulfhydryl groups may be increased above normal in leukocytes from chronic lymphocytic and granulocytic leukemia, with a markedly increased value in patients having acute lymphocytic leukemia. In the few patients studied, both alkaline and acid phosphatases tended to be low in acute leukemia. Beta-glucuronidase activity of leukocytes of chronic leukemia tended to be lower than that of normals, normal in acute lymphocytic leukemia, and elevated in acute granulocytic leukemia. Distribution of lipids may be altered in leukemic cells.<sup>88</sup> Binet, Bernard, Wellers and Mathé<sup>89</sup> have noted increased blood glutathione in acute leukemia.

*In summary, these detailed investigations of the fundamental biochemistry of the leukemic cell have served so far only to add what Valentine<sup>87</sup> aptly terms "additional biochemical parameters" to the differential diagnosis of the morphologic types of leukemia. As yet, these investigations have failed to provide a basis for rational therapy but might be found useful in following the treatment of this disease by means of serial determinations of known biochemical abnormalities.*

Competitive chemotherapy, then, appears to be nonspecific and may be limited by the relative life spans of normal and leukemic leukocytes. Ottesen and Hevesy<sup>90</sup> have reported briefly experiments which suggest that there may be two types of lymphocytes in the normal, the one having a life span of approximately one week, and the other, a life span of very much greater duration. Weisberger *et al.*<sup>91</sup> used S<sup>35</sup> labelled cystine to estimate leukocyte life span, and found total spans of approximately 13 days for normals, 8 days in chronic granulocytic leukemia, 3 days in acute leukemia, and "longer than the period of observation" (15 to 30) days for chronic lymphocytic leukemia. Using C<sup>14</sup> labelled adenine, Hamilton<sup>92</sup> studied one patient having chronic lymphocytic leukemia in whom the lymphocyte life span appeared to exceed one year! Studies on the incorporation of P<sup>32</sup> into leukocytes of patients having leukemia made in this laboratory<sup>93</sup> would indicate that the life span of the lymphocytes of some patients afflicted with acute lymphocytic leukemia is of the order of one week, in contrast to life spans probably in excess of 30 days for the lymphocytes of some patients having chronic lymphocytic leukemia. Our similar data on the life span of granulocytes in patients afflicted with chronic granulocytic leukemia show a most probable survival of the order of 3 to 5 days, in contrast to the life span of approximately nine days in the circulating blood

stream of normals reported by Kline and Clifton;<sup>94</sup> 4 to 6 days for normal leukocytes from marrow culture studies.<sup>95</sup> Marrow culture studies in this laboratory<sup>95</sup> also confirm the shortened life span of the leukemic granulocyte. Unfortunately, there is no morphologic method of identification of the postulated short-lived lymphocytes in the blood of normal or patients having leukemia.

Reference to TABLE 1 will support, only in a very general sense, the observation of Rosenthal and Harris<sup>15</sup> that the relative incidence of leukemias tends to parallel the relative frequency of cells of the same morphologic type in the blood of normals. A close correlation between the incidence of leukemia by cell type and the distribution of these cells in the normal marrow and blood would support the idea of a chance mutation providing the initial leukemic focus.

An examination of the incidence of the various types of leukemia by age, sex, and cell type indicates that unknown factors must surely modify this simple assumption. For example, it is difficult to understand why leukemia in the prepubertal child should show a small but significant (at the 5 per cent level) predominance in favor of the male child. One might speculate that the great preponderance of acute lymphocytic leukemia in children and its scarcity in the adult is a consequence of chance mutation in the relatively greater total volume of lymphocytic tissue in the child. There appears to be no rational reason why chronic lymphocytic leukemia should be predominantly a disease of aged men (and yet rare in aged Japanese males<sup>19</sup>). If the data on relative life span of lymphocytes in chronic and acute lymphocytic leukemia are not artifacts, it is not unreasonable to speculate that these diseases may actually represent disorders of different "strains" of lymphocytes, with a relative scarcity of the long-lived strain in the child, young adult, and elderly female.

The overwhelming proportion of acute leukemias in the child may represent the response of the disease to normal growth factors present in the child and absent in most adults. If this be true, then one would anticipate a much more acute form of the disease in the child 0 to 1 year in which postnatal growth rates are maximal. There are scattered clinical impressions that this may be the case, but this question appears worthy of quantitative investigation impossible with the presently available data.

The apparent preponderance of all leukemias of adults in the male sex is not understood. Hormonal factors appear unlikely to be of major over-all importance, since no differences between the prognosis for the male and female having either acute or chronic leukemia have been noted.

There is a large body of interesting fundamental research on the etiology and nature of animal leukemia which cannot be directly correlated with the human disease. The interested reader is referred to the reviews of Engelbreth-Holm<sup>96</sup> and of Furth.<sup>97, 98</sup>

In summary, it would appear that the leukemias are neoplastic diseases which may arise from spontaneous mutation or under the influence of various mutagenic agents. Such a mutation appears to be characterized by a loss of the normal ability to stop proliferation and by a tendency for early cell death. (Osgood has suggested that the early cell death is in itself a stimulus for continued proliferation by a failure to elaborate some inhibiting principle which



may be produced only by the mature cell.) Certain biochemical properties may be lost or accentuated by such neoplastic cells, but the known changes do not appear critical. *Most important, no studies of the fundamental metabolism of these cells or of the treatment with various antimetabolic agents have indicated that the neoplastic cells of leukemia are characterized by any one added unique biochemical requirement not present in the normal cells and which might be selectively attacked by a single chemotherapeutic agent without damage to the normal tissues of the patient.*

*Notes on the Clinical Picture of Acute Leukemia and its Correlation with the Clinical Pathology of the Patient*

Evaluation of therapeutic agents in the treatment of acute leukemia should be considered against the characteristic background of the clinical course of the untreated disease. The detailed description of the signs and symptoms of the disease has been adequately reviewed by Southam *et al.*,<sup>7</sup> Windeyer and Stewart,<sup>10</sup> and by such standard texts as those of Wintrobe,<sup>99</sup> Sturgis,<sup>9</sup> Forkner,<sup>3</sup> and Whitby and Britton.<sup>100</sup> The course and detailed analysis of pediatric leukemia is reviewed in the extensive monograph of Brandberg.<sup>19</sup> In this section we shall consider in detail those features of the disease which lend themselves to statistical analysis and may be useful in evaluation of treatment.

Most frequently, especially in children, acute leukemia has a sudden onset which may be signaled by such diverse symptoms as severe fatigue and malaise, fever, deep bone pain or joint pains, hemorrhagic tendencies, anorexia, weight loss, or failure to recover promptly from a simple intercurrent infection. Physical findings characteristically include pallor, often severe; enlargement of liver, spleen, or lymph nodes (usually not to the extent of chronic leukemia); cutaneous, mucous membrane or visceral hemorrhages; and, less frequently, cutaneous leukemic infiltration and hypertrophy of the gums, and mouth ulcers. Urologic,<sup>101, 102</sup> ophthalmologic<sup>103</sup> and neurologic involvement<sup>104</sup> have been reported. There may be radiological evidence of enlargement of the mediastinal nodes, associated acute pulmonary disease and, sometimes, leukemic lesions of the bones. Laboratory findings are more variable. In some instances, acute leukemia may manifest as puzzling a clinical picture as the syphilis of Osler's era.

Acute leukemia may vary in duration from death on the day of admission to survival in excess of one year. With the limited exceptions of the leukocyte count and morphology of the leukemic cells (degree of differentiation) there are no known simple guides to the prognosis for an individual case. The course of the disease may be fulminating or protracted, punctuated by one or more spontaneous remissions of symptoms and, sometimes, of morphologic evidence of the disease.

The usual progress of the patient is one of progressive deterioration with death attributed to overwhelming infection, exsanguinating hemorrhage, or what is termed "organ failure" or "generalized exhaustion." Present-day supportive management in a modern hospital includes the use of blood, antibiotics, special attention to diet and parenteral fluids, all measures which alone might tend to retard the natural course of the disease.



Bierman<sup>28</sup> has published data which indicate that antibiotics alone might tend to increase survival time in acute leukemias of childhood. Bessis and Dausset<sup>105</sup> have recommended the use of replacement transfusions in acute leukemia, and a remission has been reported following the use of a single transfusion.<sup>106</sup> Before such evidence can be unequivocally accepted as important in prolonging the survival time of patients afflicted with leukemia, it should be substantiated by carefully controlled studies on patients selected at random. It seems unlikely that as complex a procedure as an exsanguination transfusion would be used routinely on all patients, but logically might have been reserved for those who were not moribund upon admission. The scarcity of penicillin during the period of its initial use may have led to similar restrictions or, alternatively, the infection for which penicillin was given may have precipitated a "remission."

In any single series, if those "untreated" by reason of admission in a terminal condition should be placed in the "control" group, an entirely unreliable estimate of the relative efficacy of the treatment might result. Shimkin *et al.*<sup>37</sup> have published data on the treatment of acute leukemia in adults and children in which they were unable to demonstrate any effect of blood, antibiotics or radiation on the survival time of patients. In view of these findings and similar impressions reported by other authors, "untreated patients" in the analysis to follow are defined as those who have not received antimetabolic drugs or hormonal therapy, collected from those published reports in which there was some evidence that the investigator had not deleted cases from his series for a variety of reasons.

*Hematologic manifestations.* The marrow findings in acute leukemia are characterized by cellularity in excess of that usually noted in normals, and by an excessive proportion of immature cells of the involved type. There is a relative absence of the mature cells of the involved series. For the reasons previously given (pages 331-332), this condition is most likely attributable to premature cell death and not "maturation arrest" with normal survival time at an immature stage. There may be a relative scarcity of neutrophilic granulocytes which, if severe, may be associated with inability to resist infection. Megakaryocytes and thrombocytes may be reduced. There may be a relative depression of erythropoiesis.

Bernard and Mathé<sup>72</sup> have made multiple marrow punctures in the same patient having acute leukemia and have noted some discrepancies in the number and type of cells between the various sites tested. (The use of a single marrow puncture to define a "complete remission" might therefore be accepted with some reserve.) The marrow may not correlate well with the peripheral blood, but this finding should not be unexpected if extensive leukemic infiltration of primitive hematopoietic organs such as liver, spleen, and lymph nodes may also deliver cells to the circulating blood.

It is interesting that this hypercellular marrow of acute leukemia was found by Petrakis<sup>107</sup> to exhibit much higher pressure than that of patients having no neoplastic involvement of the marrow. At autopsy, replacement of fatty marrow by leukemic cells is common. Marrow punctures under these condi-

tions may encounter only soft bone substance. A pathologic fracture has been reported by Metcalfe.<sup>108</sup>

Anemia, often severe, is a usual accompaniment of acute leukemia. An analysis of the hemoglobin values of patients upon admission was made from the articles reviewed, but data are too scanty to permit definitive conclusions. These approximate normal distributions and mean values may be compared before and after treatment of the same patients. Since the patient is more likely to have a hemoglobin value after treatment which is closer to his initial value than to the mean of the treated group, it is essential to determine if a correlation exists between the pretreatment and posttreatment hemoglobin levels. The effect of such a correlation will be to reduce the magnitude of any difference between the means of the pretreatment and posttreatment series required for statistical significance.

Examination of the bone marrow would suggest that the anemia of leukemia is attributable to a "crowding-out" of erythropoietic elements. However, the careful studies of Berlin<sup>109</sup> have indicated that hemolytic anemias are common in leukemia, even in the presence of a normal hemoglobin level. This finding has also been confirmed by Brown, Elliott, and Young<sup>110</sup> and by Bernard.<sup>111</sup> Crosby<sup>112</sup> has shown that the regenerative capacity of the unimpaired marrow is so great that anemia may not be manifest until the life span of the erythrocyte is reduced from its normal value of 120 days to approximately 20 days. While grossly the leukemic marrow is not normal, it does possess a capacity for recovery if the hemolysis is slowed by adrenal hormones.

This hemolytic anemia may be a limiting factor in the patient's management. Johnson, Signorelli, and Pizzolato<sup>113</sup> describe a patient who, without gross bleeding, was given 214 transfusions of fresh blood over a 15-month course and was found to have extensive hemosiderosis at necropsy.

It may be more than coincidence that the hemolytic anemia of leukemia with a short life span of erythrocytes is noted in a disease in which leukocyte survival time is also sometimes shortened and thrombocytes frequently decreased in number. Cortisone has been noted to decrease hemolysis and to increase granulocytes and thrombocytes. Leukemia may have a common factor which produces premature death of all hematopoietic cells as well as possibly decreasing proliferation of all except leukemic cells. Such a mechanism may not be limited uniquely to leukemias. Crosby<sup>112</sup> has pointed out the triad of hemolysis, leukopenia, and thrombocytopenia in paroxysmal nocturnal hemoglobinuria. The coexistence of a hemolytic anemia, mild leukopenia, and thrombocytopenia in pernicious anemia is well known.

Leukocytosis is not a universal manifestation of leukemia. Initial leukocyte counts form skewed distributions (first pointed out for leukemia by Best, Limarzi, and Poncher<sup>114</sup>) which approximate normal distributions by the substitution of the logarithm for the leukocyte count in thousands per cubic millimeter. The median (middle) values are the typical values for such a distribution, and either the medians or the averages of the logarithms of the leukocyte counts should be compared before and after treatment. Again, a correlation analysis should be made as with hemoglobin levels, but our experience with

chronic leukemia has indicated that no correlation exists between pretreatment and posttreatment levels.

It has been the observation of Zuelzer,<sup>115</sup> Southam *et al.*,<sup>7</sup> and Shimkin *et al.*<sup>37</sup> that (for groups of patients) low leukocyte counts are associated with greater longevity, and that high leukocyte counts are associated with more fulminating forms of the disease. These differences appear to be most pronounced between patients having leukocyte counts less than 5000 and those having counts greater than 100,000, but it is likely that, as a group, patients having leukocyte counts of less than 30,000 will do better than those having counts above this figure.

From the data surveyed for this review, as many as 40 per cent of children and *ca.* 25 per cent of adults may have normal initial leukocyte counts. Not enough data could be found to permit correlation studies between the initial leukocyte count and the prognosis for survival of the individual patient.

The etiology of the hemorrhagic manifestations in leukemia is not clear. This subject has been studied intensively by Freeman and Hyde<sup>116</sup> and by Soulier and Dausset.<sup>117</sup> It would appear that a necessary condition for bleeding is a thrombocyte level below 50,000 to 60,000 per cubic millimeter. This is not a sufficient condition; numerous patients were observed not to bleed with thrombocyte levels below this critical point. Tests for heparinlike anticoagulants have demonstrated such substances, but without relationship to bleeding tendency. Both authors agree that the fibrinogen level is normal or elevated in bleeding patients. While the prothrombin time may be normal in leukemia, there is no evidence that the bleeding is related to a coagulation defect revealed by this test. It would appear that any agent which results in a significant and sustained increase in thrombocyte levels\* might have therapeutic merit. Because of the technical difficulties in obtaining reproducible thrombocyte counts, it is suggested that investigators mention the standard errors of data obtained by the method of enumeration used. Serial clot retraction studies also may be found useful in following hemorrhagic tendencies.

*Manifestations of the increased numbers of neoplastic cells.* Riddle and Sturgis<sup>120</sup> in 1927 noted the hypermetabolism which is frequently observed in the leukemias. This condition is believed to be a result of the increased metabolic demands of the leukemic cells (and possibly increased erythropoiesis) and not a result of secondary thyroid dysfunction.<sup>120, 121</sup> In the chronic leukemias, radiation treatment is followed by a decrease of this hypermetabolic state;<sup>120</sup> antithyroid drugs have little effect upon the course of the disease.<sup>122, 123</sup> Relatively little data was found on energy metabolism in acute leukemia. Serial determinations of BMR in hospitalized and "trained" adults would appear to offer an objective method of evaluation of the total effect of therapy for the individual.

Uric acid levels are frequently elevated in leukemia, both in blood and in the urine. This condition is presumably derived in part from the nucleic acids of the leukocyte and has been observed to increase following effective radiation

\* Henstell *et al.*<sup>118</sup> have pointed out that exchange transfusions of fresh blood can apparently increase circulating thrombocytes for one or two days, but cross-transfusion experiments in man of Bierman *et al.*<sup>119</sup> indicated that it is unlikely that any increase in normal leukocytes will result from such a procedure.



treatment,<sup>8</sup> urethane<sup>124</sup> and Aminopterin or cortisone.<sup>26</sup> Uric acid excretion appears to be an indicator of the effectiveness of drugs which either actually kill cells or cause a decrease in their proliferation by metabolic competitive inhibition. Uric acid stones have been noted in acute leukemia.<sup>125</sup> Total nitrogen and phosphorus balance studies<sup>126, 127</sup> may also be of value in selected instances.

Fever associated with acute leukemia may follow infection or may be only a manifestation of the general hypermetabolic conditions of the patient. A decrease in body temperature to normal levels has been noted with spontaneous remission or effective treatment. It is suggested that pyrexia be measured in some form such as degree days, the product of the degrees' elevation above 99.4° F. times the number of days of elevation.

Unfortunately, no data are available on the characteristic pattern of the measurements just mentioned. If the measurements appear to have any merit for the controlled evaluation of new agents, it is suggested that they be used in the form of serial measurements in the same patient. When the results are combined for analysis, it will be essential to determine the form of the distribution of these values and to determine whether or not a correlation exists between pretreatment and posttreatment values. Such procedures are not formidable and will result in obtaining increased information from such studies.

Organ enlargement is extremely difficult to evaluate except in a very approximate fashion. In lymph nodes one may be dealing with an approximately spherical object. A decrease in the radius by a factor of two represents a decrease in the *volume* of leukemic cells in that node by a factor of eight. An attempt to push therapy to produce a linear decrease in organ diameter with time could thus result in overtreatment and undesirable side effects.

Bone changes in acute leukemia have been studied by a number of authors including Dresner,<sup>128</sup> Silverman,<sup>129</sup> Dale,<sup>130</sup> and Karpinski and Martin.<sup>131</sup> In the experience of Karpinski and Martin, two thirds of the 33 children studied showed osteolytic lesions in the long bones on initial examination. Lesions of the skull may be infrequently noted, and soft tissue lesions of the chest have been described. The typical bone lesions are of four general types: (1) transverse bands of *increased* density near the metaphaseal ends of long bones (no adequate explanation exists from post-mortem examinations) or transverse bands of decreased density in the same area; (2) diffuse changes in trabecular pattern; (3) spotty areas of decreased density which correspond to local osteolytic lesions; and (4) periosteal reaction.

Conybeare,<sup>132</sup> Wintrobe and Mitchell,<sup>133</sup> and Bichel<sup>134</sup> have reported no radiologic or characteristic pathologic changes in the joints at post mortem, but Dresner<sup>128</sup> reports leukemic infiltrations of the synovia in one patient having rheumatoid joint symptoms.

Correlation between radiologic evidence of bony destruction and bone pain is sometimes poor. Patients may have painless advanced radiographic lesions or conversely, may have severe pain without radiologically demonstrable lesions. Further, bone infiltration may have little relationship to leukemic infiltration of organs or the leukocyte count.



Serial X-ray examinations of bony defects may offer additional information about the effectiveness of various therapeutic agents. Healing after Aminopterin has been reported<sup>128, 131</sup> but may also occur in a spontaneous remission.<sup>131</sup>

*Spontaneous remissions in acute leukemia.* Remissions have been sporadically observed in acute leukemia almost as long as the disease has been recognized. Eisenlohr<sup>135</sup> in 1878 noted the most typical pattern of remission. A 19-year-old male was seen with a remarkable leukocytosis and severe anemia (leukocytes nearly equal in number to erythrocytes), hepatomegaly, and splenomegaly. A febrile illness supervened, followed by a decrease in the leukocytosis and a regression of organ enlargement. Heuck's<sup>136</sup> patient of 1879 followed a similar course, but in this instance apparatus was available for estimating the decrease in leukocyte counts. The general subject of spontaneous remissions has been reviewed by Forkner,<sup>8</sup> Birge *et al.*,<sup>137</sup> Jiménez de Asúa,<sup>138</sup> Southam *et al.*,<sup>7</sup> and by Huth.<sup>139</sup>

Any comprehensive review of this subject is handicapped by cases buried in articles appearing under quite different titles, or in publications inaccessible during the preparation of this review. Criteria for a remission are not always identical for the various reviewers. For example, some of the cases cited by Huth<sup>139</sup> as remissions of leukemia may also be interpreted as fortunate observations of remission of the "preleukemic" phase of marrow hypoactivity described by Block, Jacobson, and Bethard,<sup>70</sup> since the diagnosis of leukemia at the time of remission was not completely established. For this reason, we have included only those cases in which there appeared to be an adequate basis for the diagnosis of leukemia prior to improvement in the patient's clinical status. Criteria for a remission are essentially those of Southam,<sup>7</sup> but we have placed no lower limit on the duration of a remission.

Since marrow aspiration is a relatively new procedure, our compilation is composed principally of "partial" remissions, *i.e.*, no evidence of the return of the marrow to a normal differential count. While instances have been described in which the diagnosis of leukemia could not have been made from blood or marrow findings during the period of remission, it remains to be established by much more exhaustive study that "nests" of leukemic cells do not persist in other portions of the marrow or in other organs of the body during such "complete" remissions.

Our analysis has been greatly aided by the comprehensive tabulation of Southam *et al.*,<sup>7</sup> to which we have added 51 cases\* from the literature to provide 102 cases for analysis. For the reasons given above, this tabulation is necessarily incomplete, but appears to form a suitable homogeneous body of data. The primary purposes of this analysis are to examine the factors associated with these remissions and to consider in detail the duration and character of remissions and thus provide some data with which drug-induced remissions may be compared.

TABLE 5 illustrates the difficulties in evaluation of the scattered data on the frequency of remissions. It would appear unlikely that Diamond and Luhby,<sup>166</sup> Southam *et al.*,<sup>7</sup> or Zuelzer<sup>115</sup> would note an incidence of approximately 10 per cent spontaneous remissions in children, and yet Brandberg<sup>19</sup> or

\* See footnote on following page.

TABLE 5  
SOME REPORTED ESTIMATES OF THE FREQUENCY OF "SPONTANEOUS" REMISSIONS IN ACUTE LEUKEMIA

Authors	Adults	Children	Total	Partial	Complete
Diamond and Luhby <sup>166</sup>		300	26	14	12
Southern et al. <sup>7</sup>		est. 83	12	6	6
	est. 67		1	1	—
Darte et al. <sup>170</sup>		37	1	—	1
Jersild and Mehlsen <sup>171</sup>		60	2	?	?
Zuelzer <sup>115</sup>		75	18	?	?
Smith and Bell <sup>168</sup>		74	3	?	3
Huth <sup>139</sup>	101		1	?	1
Rodgers et al. <sup>167</sup>		152	0	—	—
Brandberg <sup>19</sup>		140	0	—	—
Rosenthal <sup>169</sup>	2000		6	?	?
Ross <sup>169</sup>	?		Never		
Damashek <sup>169</sup>	?		1-2%		

Part of the diversity noted may be attributable to differences in definition of "remission" by the various authors.

Rodgers et al.,<sup>167</sup> would fail to note any remissions in their series of 150 cases each, unless some fundamental differences of definition of "remission" exist. Ross's<sup>169</sup> observation that he had noted no spontaneous remissions in adults emphasized the lesser incidence of this phenomenon.

TABLE 6\* indicates that remissions have been observed in acute leukemia of all morphologic types, in both adults and children and in both sexes. This table is probably not a true indication of the relative frequency of remission in various age groups or types of leukemia.

A detailed review of the factors associated with the recorded remissions leads one to suspect that these may not be "spontaneous." A summary of these factors is shown in TABLE 7. In 38 of the 76 remissions in which a definite antecedent event could be found, the remission was preceded by a febrile illness. Additional remissions were preceded by injection of foreign proteins in the form of various extracts<sup>172</sup> or by such nonspecific trauma as surgery<sup>144</sup> or eclampsia.<sup>137</sup> Dean et al.<sup>144</sup> noted transient improvement of variable duration, manifested by decreased organ size, less fatigue and anorexia, and increased granulocytes in four children subjected to thymectomy. This pattern was repeated in these same patients when, after relapse, ACTH was given. Sharnoff and Raymond<sup>173</sup> noted transient clinical improvement after injection of epinephrine for a Thorn test.

TABLE 8 summarizes the characteristics which appear to be common to "spontaneous" remissions, those induced by ACTH or cortisone and those induced by exchange transfusions. The agents certainly produce an increased incidence of "remission," but on the basis of the limited evidence at hand, the similarities in character and duration of the remission produced are remarkable. The differences in median duration of remission shown between cortisone-ACTH and "spontaneous" or transfusion remissions could occur by chance alone at least 15 per cent of the time!

\* (1) For children, references 27, 29, 35, 41, 56, 106, 139 to 149 inclusive; (2) for adults, references 23, 43, 55, 111, 135, 150 to 165 inclusive.

TABLE 6

AGE AND SEX INCIDENCE OF 102 RECORDED "SPONTANEOUS" REMISSIONS OF ACUTE LEUKEMIA

	Type of Leukemia									
	Un-classified		Lympho-cytic		Granulo-cytic		Monocytic		Total	
	M	F	M	F	M	F	M	F	M	F
Adults.....	9	5	4	2	12	4	5	7	30	18
Children.....	4	5	15	19	5	4	1	1	25	29
Both.....	13	10	19	21	17	8	6	8	55	47

The "unclassified" group includes six patients in whom sex was not given and who are classified at random for this table.

TABLE 7

FACTORS ASSOCIATED WITH "SPONTANEOUS" REMISSION

Factors	Adult patients	Children patients	Total patients
No evident antecedent*.....	17	9	26
Pyogenic infections.....	8	15	23
Fever—unknown etiology.....	1	4	5
Virus diseases			
Chicken pox.....	—	3	3
Measles.....	—	1	1
Feline leukopenia.....	—	2	2
Infectious mononucleosis.....	4	—	4
Transfusions, usual.....	8	5	13
Transfusions, exchange.....	2	1	3
Transfusions, Marrow.....	1	—	1
Various extracts			
Bone marrow.....	1	3	4
Urine or feces.....	1	2	3
Pentonucleotides.....	—	2	2
Adrenal extract.....	1	—	1
Nonspecific trauma			
Surgery, thymus.....	—	2	2
Surgery, spleen.....	—	1	1
Eclampsia.....	1	—	1
Various agents			
Nitrogen mustards.....	1	—	1
Urethane.....	—	1	1
Arsenic.....	1	—	1
B-12, folic acid.....	1	3	4
Totals	48	54	102

Only one antecedent is listed for each patient, hence another reviewer might arrive at a slightly different result by alternate choices of "most important" antecedent event.

\* Usually inadequate detail in report.

The assumption that similar phenomena arise from a common fundamental mechanism is not without hazard. With this reservation, it is proposed\* that the "spontaneous" remissions in leukemia are induced, in at least some

\* This is not necessarily an original suggestion, but the evidence presented here appears more complete than evidence previously given.

TABLE 8  
COMPARISON OF STEROID INDUCED, EXCHANGE TRANSFUSION AND SPONTANEOUS REMISSIONS

Feature	Spontaneous remission	ACTH-Cortisone*	Exchange transfusion†
Incidence (approx.)	10%	50%	22% <sup>112</sup>
Preceded by	Adrenal stress	Adrenal hormones	?
Induction time	ca. 2 weeks	Same	Same
Marrow			
Leukemic cells	Decreased	Decreased numbers and mitoses	Decreased
Normal cells	Increased	Increased	Increased
Hemolytic phenomena	Decreased	Decreased	Decreased
Hemorrhage	Decreased	Decreased	Decreased
Organ size	Decreased	Decreased	Decreased
Fraction complete (approx.)	50%	50%	50%
Predominant cell type of leukemia	Lymphocytic	Lymphocytic children	Unknown
Duration in weeks			
Median (95% confidence limits)	6.6 (5.3-8.2)	5.2 (4.1-6.6)	4.9 (3.9-6.2)
95% range	1-58	1-56	1-33
Patients	102	77	39
Probability of chance difference of medians	15%		76%

The tabulation of cases for steroid or transfusion remissions is not intended to be complete but is believed representative. Duration of remission appears to follow log-normal distributions ( $P(\chi^2)$  steroid curve 28%, 4 d.f.).

\* 7 adults and 70 children. From references 41, 127, 174, 175, 176, 177 and 26, 29, 32, 41, 127, 173, 175, 178, 179, 180, 181, 182.

† From the review of Bessis and Dausset.<sup>105</sup>

instances, by a variety of causes of adrenal stress which, in turn, produces an increased output of adrenal cortical hormones at a level adequate to influence the course of the disease. The action of replacement transfusions may be postulated as a direct transfer of ACTH and/or "cortical steroids" or, more likely, a stimulus for its endogenous production in the recipient. Alternatively, these very similar durations of remission may represent nothing more than the upper limit of the ability of the patient having acute leukemia to respond to any agent.

From the available studies on the detailed nature of remissions, these improvements begin with a gradual loss of fever, anorexia, and malaise, followed by a decrease in organ size, depression of the elevated leukocyte count (sometimes to leukopenic levels), and increase in normal leukocytes, reduction of hemorrhagic phenomena, and lessened requirements for transfusions. In some instances, there has been reported a return of the marrow to an apparently normal status.

If the mechanism of remission be through the production of endogenous adrenal hormones, some of the known actions of these hormones are: (1) decrease in the proliferation of neoplastic cells with increase of normal cells (Cramer<sup>183</sup>); (2) gradual improvement in the acquired hemolytic anemia; (3) rather prompt increase in thrombocytes and granulocytes; and (4) production of euphoria and lessened symptomatic distress.

The biochemical basis for these actions is unknown. The speed of response will depend upon the life span of the cells affected by the agent, e.g., ca. one



week for thrombocyte response; sometimes four weeks or more might be required to observe a marked decrease in the hemolytic anemia.

The duration of "spontaneous" remissions is extremely variable and is approximated by a log-normal distribution.\* The median (logarithmic mean) duration in 54 children was 5.2 (4.1 to 6.6) weeks, 9.4 (6.2 to 14.3) weeks in 48 adults. The probability of a chance difference of these median values is greater than 1 per cent, hence a difference of median duration between remissions in adults and children may exist.† However, the combined data for adults and children form a homogeneous distribution with median survival of 6.6 weeks, and this combined estimate is considered preferable until more data to test age differences become available. The 95 per cent range of these remissions will vary from less than one week to greater than one year. An investigator should accordingly feel no hesitancy in reporting *definite hematologic and clinical transient improvement of less than one week's duration as a "remission."* (Four per cent should be < 1 week, 14 per cent < 2 weeks.) In the presence of any of the factors associated with "spontaneous" remissions, he might be properly hesitant in attributing *an isolated instance* of a remission of one year's duration to the action of a new therapeutic agent.

Bernard<sup>111</sup> relates a case report which emphasizes that an unusually long remission is not unexpected from such a skewed distribution of duration of remissions. A young adult male was seen with typical severe acute leukemia, given only necessary daily transfusions of *ca.* one liter/day for three weeks, after which a complete clinical and hematologic remission followed. He returned to the strenuous occupation of a ballet dancer for three years and eight months! After relapse, death occurred in less than one week. It appears important that blood transfusions from this patient while in remission failed to influence the course of other patients with acute leukemia.

The complications of acute leukemia would lead to rather frequent examples of adrenal stress similar to those of TABLE 8. It is remarkable that so few remissions have been recorded. It seems possible that the relatively greater number reported by Diamond<sup>166</sup> in recent years may be a result of control by the presently available antibiotic agents of stress-producing acute infections which formerly would have killed the patient before a remission could result.

Multiple spontaneous remissions have been noted as well as under the adrenal hormone therapy, but one gains the general impression from the literature that these are much less frequent, and usually of shorter duration, than the primary remission. Finally, the patient fails to react as formerly, and death ensues. The animal experiments of Burchenal<sup>184</sup> would suggest that one possible mechanism of such resistance is the production of a resistant mutant variety of the original neoplastic cell.

In summary, while the individual remission is highly unpredictable as to its occurrence or duration, remissions similar in duration to those occurring without definitive treatment may be analyzed precisely by the maximum

\*  $P(\chi^2)$  53 per cent, 6 d.f. This homogeneity is the best evidence that the widely spaced cases assembled are representative of the same disease process.

† Similar tests could not be made for steroid or exchange transfusion remissions with the limited number of cases available.

likelihood methods to be demonstrated in the sections to follow, even before all of the patients under therapy have relapsed.

*Duration of survival.* It is believed that the most objective single measurement of the effectiveness of total therapy is the duration of survival. This period may be measured from both the time of diagnosis and the onset of first persistent symptoms relevant to leukemia. The over-all pattern of survival times in untreated leukemia, as in a wide variety of benign and malignant diseases, appears quite characteristic but is highly variable from one patient to the next.

In view of differences in setting the date of onset of the disease (if survival is measured from first symptoms) or variation in referral policies which could affect the time at which the patient is first treated, the duration of life of treated patients can be most appropriately compared with the duration of life of similar untreated patients seen in the same institution. If such a series can be assembled from the relatively recent past years in which comparable adjuvant therapy such as blood and antibiotics were available to physicians of equal experience for the supportive treatment only of patients afflicted with acute leukemia, then the survival of such patients forms the best "control" available for comparison with the treated group. It is equally important that there has been no change in the diagnostic criteria of the institution between the control and the treatment period.

In some institutions no such comparable control group will be available for a variety of reasons, not the least of which is the division of the relatively few patients having acute leukemia among internists, pediatricians, and radiologists. This section attempts to provide an estimate of the typical survival time of untreated patients having acute leukemia for such investigators who have no personal control series. To accomplish this, it has been necessary to collect cases from a large variety of sources in the literature. Such a collection is not necessarily a clinically homogeneous sample and may be seriously biased in some instances by selective reporting not evident to the reviewer. In many instances, survival time was reported inadequate in detail for most precise analysis. Further, inadequate data were found to compile statistics for "all types" of leukemia in which the number of cases of each cell type followed the relative proportions shown in the early sections of this paper. *The resultant data should be taken as approximations. It is hoped that these findings may be superseded by appropriate reports from large centers in which uniform criteria of uniformly excellent supportive therapy have been given to large groups of patients and analyzed in detail for each of the major types of leukemia, separately reported by adult and child classifications.*

A plot of the number of patients dying in successive monthly intervals will form a skewed distribution. This apportionment becomes a normal or Gaussian distribution by the substitution of the logarithm of the survival time for the time in weeks, months, or years. Since the common tests of statistical significance require that the data evaluated be derived from normal distributions, average survival times are inappropriate measures of survival in leukemia. In a normal distribution, the patient represented by the average

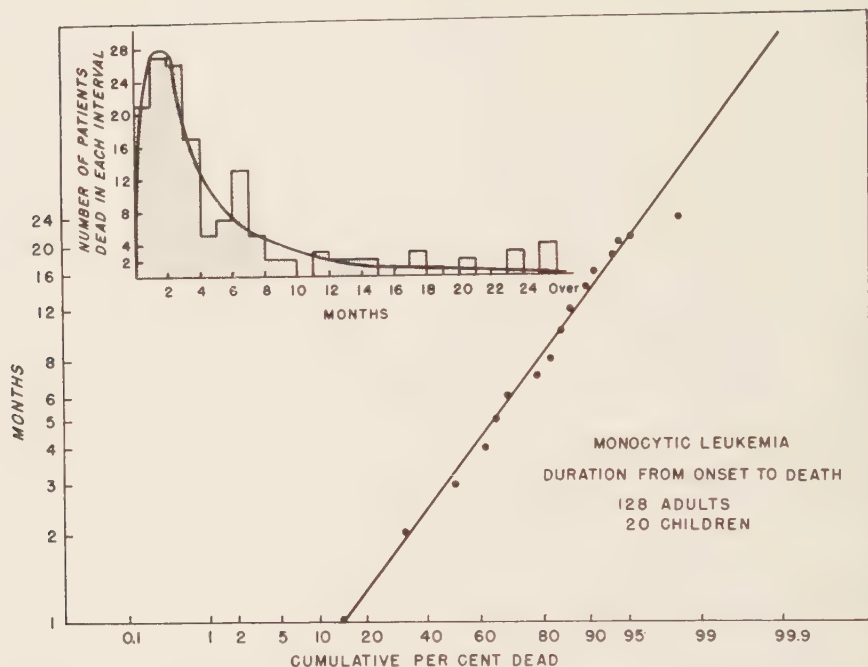


FIGURE 2. Duration of total survival from first symptoms to death in monocytic leukemia in both 20 children and 128 adults. Data were assembled from references 19, 33, 185, 186, 187, 188 and 23, 164, 186, 188 through 198, 200 through 207.

is the middle patient in the series if the patients are ranked in order of survival. For such skewed distributions of survival times as are found in acute and chronic leukemia (and all types of untreated, persistent, or recurrent carcinoma tested), an average survival time is likely to represent not the middle patient in order of survival, but about the 65th to the 70th patient of a series of 100. Average survival times are thus not only inefficient (their use could mask significant differences in results of therapy) but are misleading as well and should not be used unless duration of life in *treated* patients is found (by  $\chi^2$  or similar tests) to follow a normal distribution.

An effort was made to limit the data collected for this review to the period in which antibiotics and blood were available as adjuncts but, in some instances, it was necessary to go back to 1937 to obtain minimal quantities of data. In the case of monocytic leukemia, the compilation of 148 cases probably contains the majority of all separately recorded cases in the literature and covers a much wider time period. In view of the heterogeneous nature of the sources of data, a simple graphical method of analysis was utilized which has been previously described.<sup>24</sup> One of the resulting distributions is illustrated in FIGURE 2 but to save space, only the final results of such computations are given in TABLE 9.

For each assembled series, data were zoned over seven time intervals which were approximately equal on a logarithmic time scale.\* Chi-square analysis

\* Such zones represent a compromise. An ideal logarithmic time scale would be unreasonable in view of the

was used to test the over-all homogeneity of the resulting compilations by tests of correspondence between the observations and the fitted curves. (In most instances the individual contributions to each series were too small to permit tests of homogeneity among authors.) Probabilities that chance fluctuations alone would produce as much discrepancy between the fitted curve and the observations as was actually observed varied from 10 to 80+ per cent (3 or 4 d.f.) for the various groups which contained more than 50 entries. Thus despite differences in geographical location of the source material and time over which the data were collected, it would appear that the resulting compilations approximate homogeneous groups to an extent adequate for clinical purposes.

The only marked differences in the duration of survival between adults and children appear in total duration from onset of symptoms to death and in onset to diagnosis for combined data on all types of leukemia. (For adults, monocytic leukemia dominates these totals. Clinically, these comparisons are suspect.) While the median total survival from onset to death appears to be slightly greater in the adult than in the child, there is also a greater median time from onset of symptoms to diagnosis. In view of this disparity and the probability that the difference between a median survival time of 2.7 months in children\* and a median survival time of 3.3 months in adults could arise if the true medians were in fact identical in at least one series in 38 of this very large size, it must be concluded that no difference in prognosis for survival between the adult and child afflicted with acute leukemia is demonstrated by these data. (Even if one chooses to consider the indicated differences as "significant," the clinical importance of a 0.6 month difference in median survival appears negligible.) "Not proven" is not identical with "not true," and significant and important differences might be demonstrable with better data or from the action of various therapeutic agents.

No data were available which would permit a correlation analysis of the duration of time from onset of symptoms with the duration of survival after therapy. Such a study for patients having chronic leukemia<sup>218</sup> treated in this department failed to demonstrate a significant correlation, and it may be suspected that the same situation obtains in acute leukemia. Under such circumstances, the median duration of survival before treatment cannot be added to the median duration after treatment to obtain the median total duration.

The logarithmic standard deviations of the distributions summarized in TABLE 9 were deleted in order to reduce the complexity of the table. For leukemia, acute or chronic,<sup>218</sup> and for a wide variety of carcinomas,<sup>219</sup> these deviations will have a value not widely different from 0.400. This similarity has an important implication. The central 95 per cent range of the survival times in any series is proportional to the median survival time for that series. This range of survival times may be mentally approximated from any given median by dividing the median by 6 to obtain the lower limit and by multiply-

approximate nature of survival data recorded predominantly as one month, two months, etc. This severely limits the precision with which correspondence between calculated curves and observations may be tested. More critical examination of survival distributions depends primarily upon better data.

\* A previous estimate of 3.9 months median duration of survival for 218 children is based upon a more homogeneous group and is probably to be preferred as "base-line" data.



TABLE 9

RESULTS OF ANALYSIS OF AVAILABLE DATA ON PATIENT DELAY, SURVIVAL FROM DIAGNOSIS TO DEATH AND TOTAL SURVIVAL FROM ONSET TO DEATH\*

Item**	Type of leukemia	Children					Adults					
		No. of Pts.	P(χ) <sup>2</sup>	d.f.***	Median survival (conf. limits)	Range of central 2/3	Prob. off chance diff.	No. of Pts.	P(χ) <sup>2</sup>	d.f.***	Median survival (conf. limits)	Range of central 2/3
Total duration—onset of symptoms to death												
1	Lymphocytic	171	24	[3]	3.6 (3.2-4.0)	1.6-7.9	59%	18			3.8 (2.6-5.7)	1.4-9.0
2	Granulocytic	41			4.2 (3.2-5.5)	1.6-10.1	30%	28			5.2 (3.8-7.1)	2.2-11.8
3	Monocytic	20			2.6 (1.6-4.2)	0.8-7.8	50%	128	>80	[4]	3.1 (2.6-3.7)	1.0-8.5
4	All types††	572	43	[4]	2.7 (2.5-2.9)	1.0-6.9	2.6%	179	11	[4]	3.3 (2.8-3.9)	1.0-9.8
Diagnosis to death												
5	Lymphocytic	18			3.5 (2.3-5.4)	1.3-9.0	3.2%	13			1.4 (0.7-2.9)	0.4-5.2
6	Granulocytic	12			1.2 (0.6-2.4)	0.3-4.1	20%	28			2.0 (1.4-2.9)	0.7-5.4
7	Monocytic	3			qns		—	33			2.1 (1.5-2.9)	0.8-5.6
8	All types††	37			2.4 (1.8-3.3)	0.4-6.2	11%	74	73	[4]	1.7 (1.3-2.3)	0.5-6.0
Onset to diagnosis												
9	Lymphocytic	40			1.2 (0.9-1.7)	0.5-3.1	43%	18			1.6 (0.9-2.8)	0.44-5.3
10	Granulocytic	30			1.2 (0.9-1.7)	0.5-3.0	5.2%	33			1.9 (1.4-2.6)	0.7-4.7
11	Monocytic	5			qns		—	35			1.8 (1.3-2.5)	0.6-4.8
12	All types††	142	80	[4]	1.2 (1.0-1.4)	0.50-2.9	0.7%	88	90	4]	1.7 (1.4-2.1)	0.63-4.8

\* All times are in months. Confidence limits define the range within which median values would be found in 95 of 100 series of patients having acute untreated leukemia of size and composition similar to that analyzed.

\*\* References used were, for children: Item 1—19, 23, 27, 28, 56, 142, 208; Item 2—19, 23, 31, 33, 35; Item 3—See FIGURE 2; Item 4—Items 1 and 2 plus 115, 144, 167, 185, 187, 188, 209, 210; Item 5—19, 23, 27, 56, 142; Item 6—23, 31, 35; Item 7—187, 188, 209, 210, 213, 227; For adults: Item 1—19, 23, 102, 153, 214, 215, 216, 217; Item 2—23, 31, 43, 113, 154; Item 3—See FIGURE 2; Item 4—Items 1, 2, and 151; Item 5—23, 153, 215, 217; Item 6—23, 31, 43, 113, 154; Item 7—23, 164, 186, 189, 190, 191, 192, 193, 194, 195, 196, 198, 201, 203, 204, 205, 206, 207; Item 8—Items 5, 6, and 7; Item 9—23, 38, 211, 215, 216, 217; Item 10—23, 31, 34, 41, 113, 154, 211; Item 11—21, 23, 26, 164, 186, 189, 190, 191, 192, 193, 194, 195, 196, 198, 199, 201, 202, 203, 206, 207, 211; Item 12—21, 23, 26, 31, 34, 38, 41, 43, 113, 154, 164, 176, 186, 190, 191, 192, 194, 195, 196, 198, 199, 202, 203, 206, 207, 211, 215, 216, 217, 228.

† Probability of chance differences between medians as great as those shown.

†† Includes cases in which no diagnosis was specified, hence total may exceed preceding 3 entries.

\*\*\* P(X)<sup>2</sup> is the probability that differences between log-normal curves used and observed values are attributable to chance fluctuation. d.f. = degrees of freedom = (number of zones with >10 entries—2).

ing the median by 6 to obtain the upper limit. In its simplest form, a log-normal analysis of a given series may thus be roughly approximated by counting down the series in order of survival to the middle patient, and then by multiplying and dividing his survival time by 6 to obtain the 95 per cent range of the distribution of all such patients.

Examination of FIGURE 2 will indicate that the majority of patients having acute leukemia will die relatively soon after onset of the disease (or diagnosis), but a few will live for periods that are astonishingly long in comparison with the periods survived by the majority. The patient who dies soon after entry into the treatment series is not necessarily a therapeutic failure, and the patient who lives an extended period of time is equally not a therapeutic triumph. Both are expected manifestations of the disease and should be included in all series.

The few patients of unusually long survival can introduce a complication into the evaluation of new therapeutic agents by delaying publication on the other patients long since dead. This difficulty may be obviated by the use of the maximum likelihood method of predication of the ultimate outcome of a series containing living patients who will ultimately die of their disease. This technique was developed by D. E. Lea<sup>220</sup> and is not unduly complicated. After a period of practice manipulation of the method, an estimate of the ultimate outcome of such a series should be obtainable in less than one half day, a short period in comparison with the weeks or months of waiting involved for the last few patients of the usual treatment series. An elegant extension of this technique to the calculation of the proportion of patients cured of carcinoma has been developed by J. H. Boag<sup>219</sup> and should materially shorten the time required for the precise evaluation of results of cancer therapy. With both methods, confidence limits of the ultimate result can be calculated, and reliable comparisons of two or more series can be obtained readily.

TABLE 10 illustrates two successive calculations of median survival time in a small series of 25 successive patients having acute and subacute leukemia treated with continuous cortisone followed by added P<sup>32</sup> after cortisone relapse. In the 16-month interval required for six of the seven originally surviving patients to die, there was no change in the predicted median (log-mean) survival time, and with only one patient living (at 45 months) the observed and predicted median survival times are essentially identical. The prediction of median survival time of 45 children treated with 6-mercaptopurine\* (Burchenal *et al.*<sup>42</sup>) appears in reasonable agreement with the ultimate result for this series.<sup>221</sup> Similar reliability of predition has been noted in the successive analysis of survival times in patients having chronic leukemia.<sup>218</sup> A simple comparison between the median values and confidence limits of TABLE 10 and the results in TABLE 9 will lead immediately to the conclusion that these methods of treatment represent significant prolongation of survival time over that observed in the cases reviewed for this article.

The methods of analysis described above, or the use of the simple log-normal methods of analysis are adequate and effective if the survival times of patients

\* The median duration of 25 6-mercaptopurine remissions<sup>42</sup> observed, 5 weeks, is not statistically different ( $P = 0.05$ ) from the duration of spontaneous or cortisone-ACTH remission medians of 5.2 weeks.

TABLE 10

SUCCESSIVE CALCULATIONS OF TOTAL DURATION OF SURVIVAL IN SMALL SERIES OF PATIENTS WITH ACUTE AND SUBACUTE LEUKEMIA

Author	Date	Patients		Treatment	Calc. median months	Obs. median (incl. living)	95% Conf. limits of est. median	s Log
		Total	Living					
Osgood <sup>66</sup>	12/52	25	7	Cortisone + P <sup>32</sup>	7.4	7.5	5.2-10.4	0.37
	3/54	25	1		7.0	7.0	5.0-9.7	0.36
Burchenal <sup>42</sup>	3/53	45	23	6-MP + antifolics + steroids	12.0	6.8	9.6-15.1	0.28
	5/54	45				12+ <sup>221</sup>		

treated follow the log-normal distribution. Our experience with a limited series of patients has indicated that an *apparent* effect of therapy with multiple agents is a proportionate increase in the survival of all patients so that the survival curve for the treated group appears to parallel the untreated group, but at a higher level. This is the effect that one would anticipate if the life span of nearly all patients could be increased by some arbitrary percentage of their survival time without treatment. If such a situation truly obtains (and this assumption needs testing by those having more complete data), then it must be attributed to the multiple agents now available for treatment of acute leukemia, since no one agent appears effective for all patients.

For patients treated with a single agent, this conclusion is not necessarily true. Steinberg<sup>222</sup> has cogently pointed out that the survival curves of Farber's patients treated with antifolic drugs do not follow the log-normal distribution. Steinberg has attributed this finding in part to the selective action of the antifolic acid drugs which produce a remission in only some of the patients. To test this hypothesis, the following statistical experiment was performed. Survival time data for each of 100 hypothetical children having leukemia were calculated from a previously published report.<sup>21</sup> An estimate of duration of first remissions in 47 Aminopterin-treated children<sup>21, 30, 39, 40, 45, 169, 198, 211, 213, 223</sup> was made which indicated a log-normal distribution ( $P \chi^2$  80 per cent, 3 d.f.) with a median duration of 9.3 weeks (6.9 to 12.5) with a 95 per cent range of 1.2 to 74 weeks. It was assumed that 51 per cent of children would show an Aminopterin remission; 7 per cent, a spontaneous remission.<sup>224</sup> From the curves for duration of Aminopterin and spontaneous remissions in children, the time of each of the postulated 58 remissions was calculated. We have the additional clinical impression that antifolic agents will require at least three weeks to act. On this basis, by the use of a table of random numbers, each of the 58 derived durations of remission was assigned to one of the 95 "untreated" survival times in excess of one month. The new survival times (for 58 patients) representing the total untreated survival time plus a randomly assigned single Aminopterin or spontaneous remission were added to the 42 unchanged survival times and then analyzed by the usual graphic method. Line *b* of FIGURE 3 resulted.

This broken line indicates that there is a transient decrease in the rate of dying, but as the therapy becomes ineffective, then the death rate once more

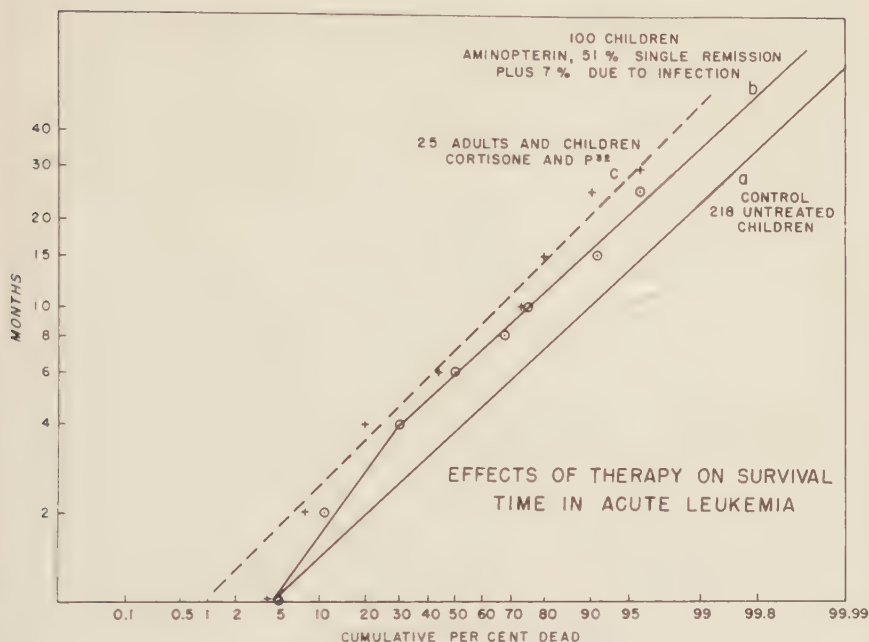


FIGURE 3. Effect of therapy on survival time.

becomes approximately parallel to the original estimate. It is of interest that this same type of pattern is apparently followed by patients afflicted with hypertension treated by sympathectomy<sup>225</sup> in comparison with a log-normal death curve (similar to line *a*) for the untreated controls. Such a break in the straight line of a log-normal distribution indicates that the course of the disease has been altered by therapy and carries the same implications as a generalized increase in survival time. It must be emphasized that this line *b* is *not intended as an estimate of the effects of Aminopterin on survival* but only to indicate a possible complication in the analysis of results.

For such data, the use of log-normal distributions is inappropriate. A curve of survival time can be made conveniently using the method of Berkson and Gage.<sup>226</sup> This curve can then be compared with the untreated controls by the use of  $\chi^2$  analysis. It is suggested that the median survival time, together with its confidence limits be reported as an index of the over-all result of such therapy.

In summary, within the limitations of precision of the basic data available for review, a relatively uniform pattern of the survival time of groups of patients having acute leukemia has been indicated. Analysis fails to disclose any important differences in the prognosis of the adult or child, or among the various types of leukemia. Relatively simple and mathematically powerful methods of analysis have been given for handling survival time or duration of remission data. Used with appropriate caution, these methods may aid materially in the evaluation of the effects of therapy. Illustrative application



of such techniques to the scattered results presently available would indicate that the prognosis for survival of the patient afflicted with acute leukemia is now probably at least double that of the period before antimetabolic and hormonal agents became available. A definitive appraisal of improvement in the treatment of leukemia awaits the detailed publication of the results of uniform treatment of a large group of unselected patients, either by a single investigator or through the cooperative efforts of many.

### *Summary*

A review has been made of relevant available publications on acute untreated leukemia.

Estimates on the incidence and prevalence of acute leukemia, by age, sex, and cell type have been presented.

The clinical features and laboratory findings have been reviewed with the intent of providing features which might be useful in the evaluation of new therapeutic agents. Available base-line data are presented.

A total of 102 spontaneous first remissions in leukemia have been analyzed. Such remissions can occur at all ages, in both sexes, and in all common types of leukemia. These remissions most frequently follow an incident which could be interpreted as "adrenal stress" and clinically appear to resemble in character those produced by ACTH or cortisone. The median duration of such spontaneous remissions is 6.6 weeks, statistically not different from a median duration of 5.2 weeks for 54 cortisone-ACTH remissions reviewed. It is suggested that the mechanism of spontaneous remissions is through the endogenous production of "therapeutic" quantities of adrenal hormones.

The duration of survival time from onset of disease to diagnosis, from diagnosis to death, and total duration from first symptoms to death was analyzed for several hundred cases in both adults and children. There is inadequate evidence to establish that the prognosis differs between the adult and child having acute leukemia of any cell type. Typical median durations found are: onset to diagnosis, *ca.* 1.5 months; diagnosis to death, *ca.* 2.2 months; onset to death, *ca.* 3 months.

Statistical analysis of data on duration of remissions and duration of survival time is discussed and appropriate recommendations are made.

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## CLINICAL STUDIES ON 6-MERCAPTOPURINE\*

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Profiting by the studies of Elion and Hitchings,<sup>1, 2, 3, 4</sup> of the Wellcome Research Laboratories, and of Stock, Clarke, Philips, Brown, and others of the Sloan-Kettering Institute,<sup>3, 5, 6, 7</sup> the Chemotherapy Service of Memorial Center has had the opportunity to evaluate 6-mercaptopurine in 269 patients having various forms of neoplastic disease.

6-Mercaptopurine has been administered almost entirely by the oral route, the usual dosage being 2.5 mgm./kg. of body weight once daily.<sup>8</sup> At this level, in children, we have seen almost no evidence of toxicity. At higher doses in children or, occasionally, at this dose in adults, evidence of marrow depression and gastrointestinal disturbances have been noted. On the other hand, in many patients, we have administered 5 mgm./kg. and even higher doses without toxic manifestations, and one patient had increasing dosage until finally a level of 12.5 mgm./kg. was given daily for a period of a week with no apparent ill effects. Since most of the patients who were given these very large doses had previously been treated with the ordinary therapeutic level for varying periods of time, it is conceivable, however, that they may have developed some tolerance to the drug.

On the question of dosage schedule we have always given a single daily dose, and we have no information at the present time as to whether there might be an advantage in giving the drug by a fractionated technique either three times daily or as often as every two hours. It is also possible that a high loading dose of 5 to 10 mgm./kg. for one to two days might decrease the usual 3- to 8-week latent period before the beneficial action of this drug becomes apparent.

Despite the fact that Philips *et al.*<sup>6</sup> and Clarke *et al.*<sup>7</sup> have demonstrated that mercaptopurine has a hepatotoxic effect in dogs, we have seen no definite clinical evidence of liver damage which could be ascribed to this drug, although an occasional case of what appeared to have been serum hepatitis has occurred in patients under therapy who have previously had transfusions. In some patients having acute leukemias with high total leukocyte counts, we have seen a rapid fall in white count after a relatively short period, 8 to 10 days, of mercaptopurine therapy. Whether this change represents a toxic or purely a therapeutic effect of the drug cannot be stated definitely at this time.

Metastatic carcinomas, sarcomas, Hodgkin's disease, lymphosarcoma, and chronic lymphocytic leukemia have not responded to 6-mercaptopurine in our experience. There has been some decrease in size of metastatic nodules of

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patients having diffuse reticulum cell sarcoma and an inhibition of the growth of osteolytic metastases as noted by a decrease in hypercalcuria in one patient treated with mercaptopurine and azaserine.

6-Mercaptopurine produced remissions in 11 out of 12 patients afflicted with early chronic myelocytic leukemia. FIGURE 1 shows a typical patient having chronic myelocytic leukemia treated with mercaptopurine with a gradual fall in white count to normal levels, rise in hemoglobin, and decrease in splenomegaly. It will be noted that maintenance therapy had to be employed in this patient, and that when, after 6 months of remission, treatment was stopped for one month there was a rapid rise in white count. This increased count fell again when treatment was reinstituted. This need of maintenance therapy has been demonstrated in most of our patients having chronic myelocytic leukemia, and one 65-year-old patient had seven remissions over a period of 13 months, following separate courses of mercaptopurine. Occasionally, however, one encounters a patient having chronic myelocytic leukemia in whom therapy can be discontinued once a remission is well-established, and in whom the remission will continue for long periods of time without further therapy. FIGURE 2 illustrates such a situation.

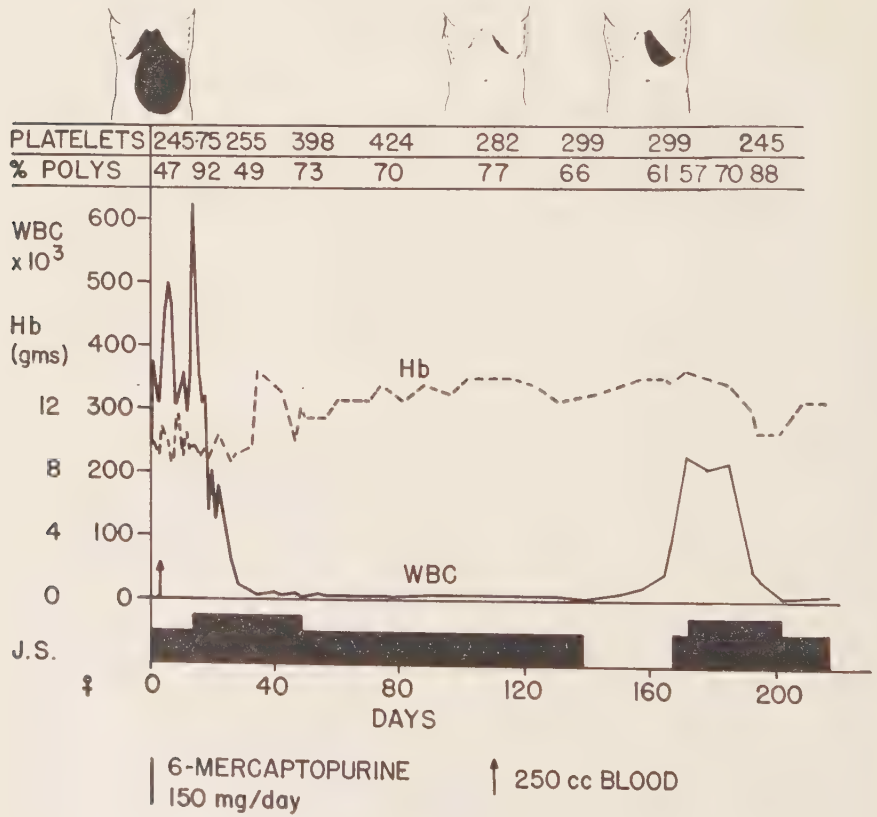


FIGURE 1

In the terminal acute stage of chronic myelocytic leukemia, mercaptopurine also seems to have some beneficial effect in contrast to most of the other therapeutic agents presently employed in treating this disease. In two out of six such patients, temporary remissions lasting from one to four months were achieved with mercaptopurine. FIGURE 3 is an example of such a response, with a decrease of the blast forms in the marrow from 80 to 10 per cent, and rises in hemoglobin, in platelets, and in the percentage of the polymorphonuclear leucocytes. As can be seen, the remission was of short duration, and a relapse occurred despite the continuation of therapy. How 6-mercaptopurine will compare with the more conventional agents such as X ray,  $P^{32}$ , urethane, triethylene melamine, or Myleran, in the treatment of chronic myelocytic leukemia, it is impossible to state at the present time. It does appear, however, that most patients respond well to this drug for periods of at least 12 months with minimal or no toxic effects.

6-Mercaptopurine is a representative of one of three classes of agents, the folic acid antagonists, the purine antagonists, and the steroid hormones, which are active against acute leukemia. It will produce remissions in acute leukemia both in children and in adults. Of 87 children having acute leukemia, 41 had good clinical and hematological remissions, 16 had partial remissions, and 30 were considered failures (TABLE 1). By a good clinical and hematologic remission is meant a marrow in which the total of leukemic cells and lymphocytes decreased to a level of less than 30 per cent; the normal erythroid and myeloid elements increased to at least 70 per cent; megakaryocytes reappeared; the peripheral blood values became essentially normal; and the patient returned temporarily to good health. FIGURE 4 shows a typical example of such a remission. It is to be noted in this group that good remissions were achieved in 10 out of 30 children whose disease had previously been shown to be resistant to A-Methopterin, and 11 out of 28 whose disease had become resistant to cortisone. Many children whose disease responded at first and then became resistant to mercaptopurine responded to a subsequent course of Methotrexate (A-Methopterin) or cortisone. Fourteen out of 37 such patients responded to Methotrexate.

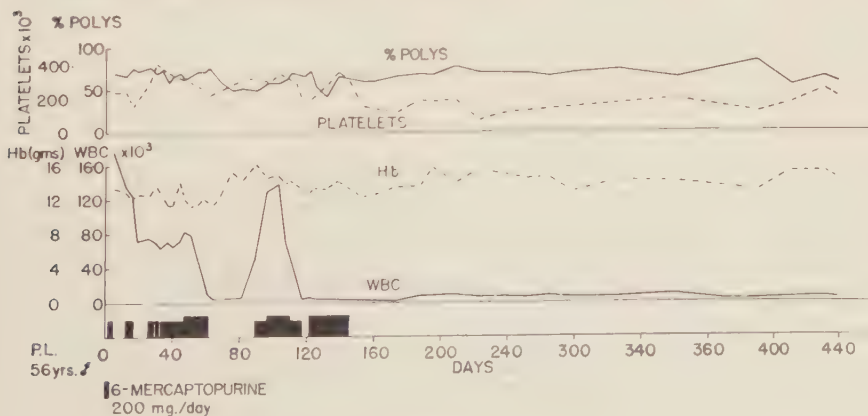


FIGURE 2



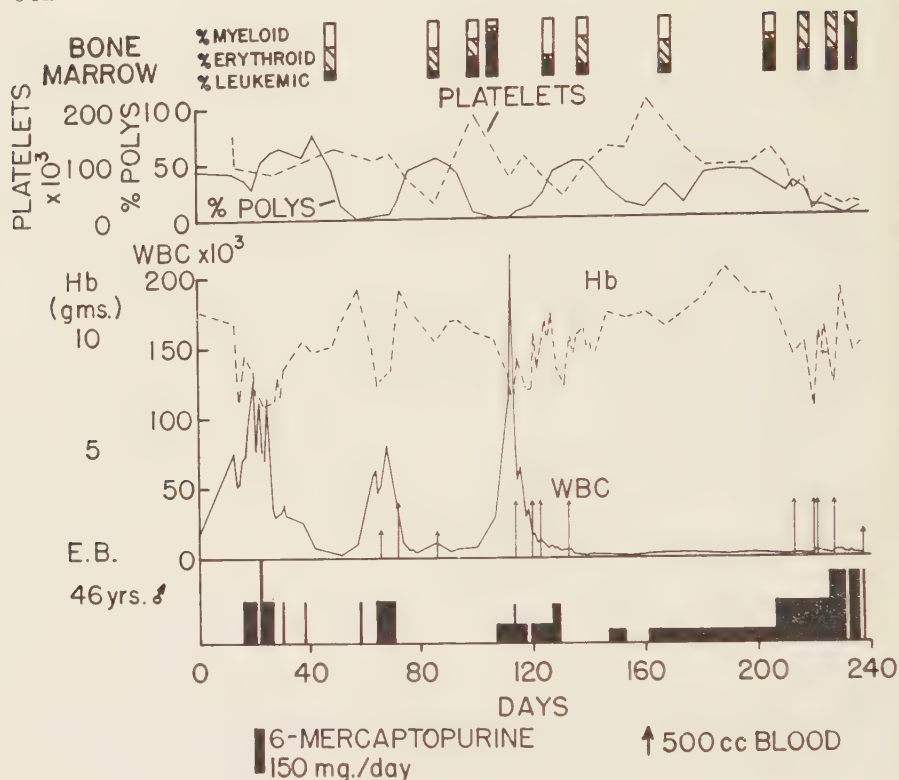


FIGURE 3

TABLE 1  
RESULTS OF 6-MERCAPTOPYRINE THERAPY IN ACUTE LEUKEMIA

	Children	Adults
Total .....	87	50
Good clinical and hematologic remissions .....	41	7
Partial remissions .....	16	10
Failures .....	30	33

It can be seen from TABLE 1 that remissions were also produced in the acute leukemias in adults by 6-mercaptopurine, although the results were not as satisfactory as in the children. In 50 patients, 7 good clinical and hematologic remissions, 10 partial remissions, and 33 failures were achieved. FIGURE 5 shows a typical good remission, which was achieved in a 21-year-old housewife with this drug. A relapse occurred in nine months despite maintenance therapy. Even myelomonocytoid and true monocytic leukemias occasionally responded to this agent. FIGURE 6 shows a 52-year-old woman afflicted with myelomonocytoid leukemia who was kept in remission for over a year on

constant maintenance therapy but is now beginning to relapse despite continuation of therapy.

The remissions in acute leukemia treated with 6-mercaptopurine lasted from 1 to 12 months in our experience, generally longer than with the steroids and a somewhat shorter time than with A-Methopterin. Although occasionally, particularly in the high-count leukemias, a fall in total leukocyte count occurred within 10 days (FIGURE 7), usually 6-mercaptopurine required 3 to 8 weeks to exert its beneficial effects, a latent period comparable to that of the folic acid antagonists but considerably longer than in the case of the steroids. In most cases it was necessary to produce a moderate leukopenia before a return of normal cells was observed.

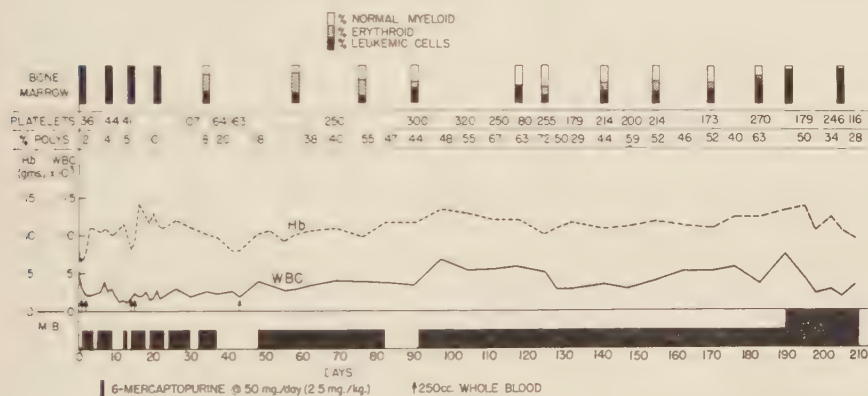


FIGURE 4

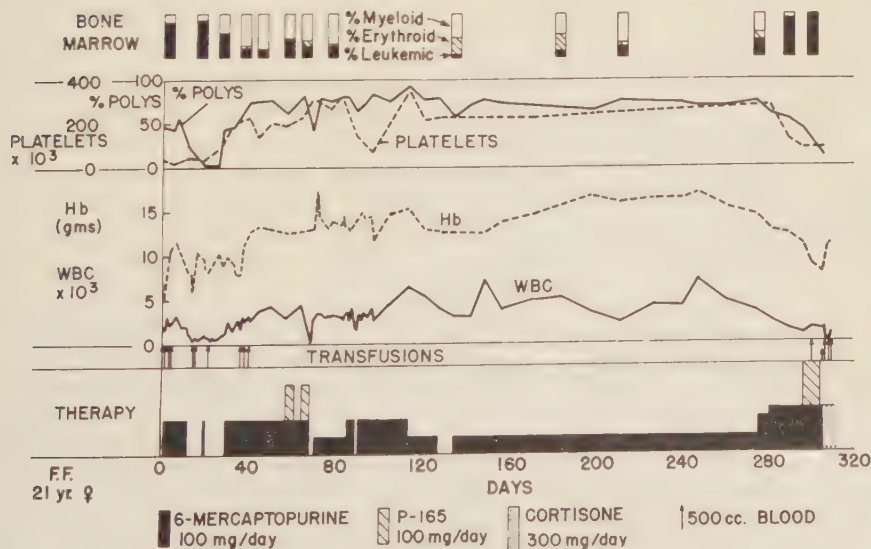


FIGURE 5

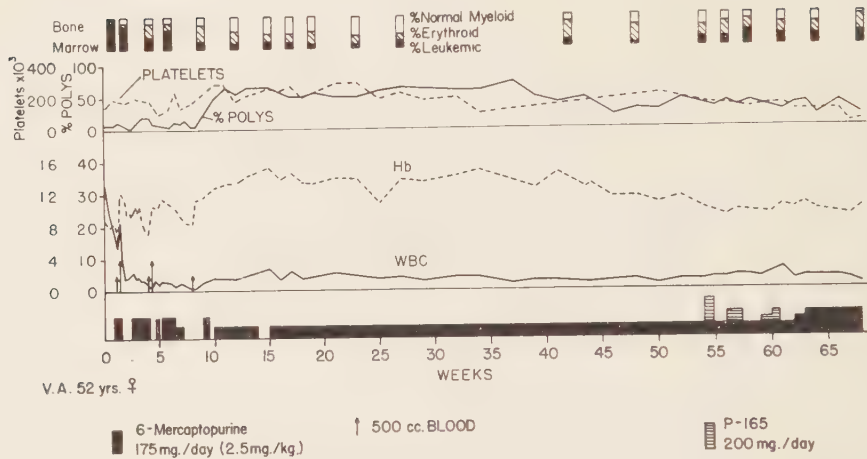


FIGURE 6

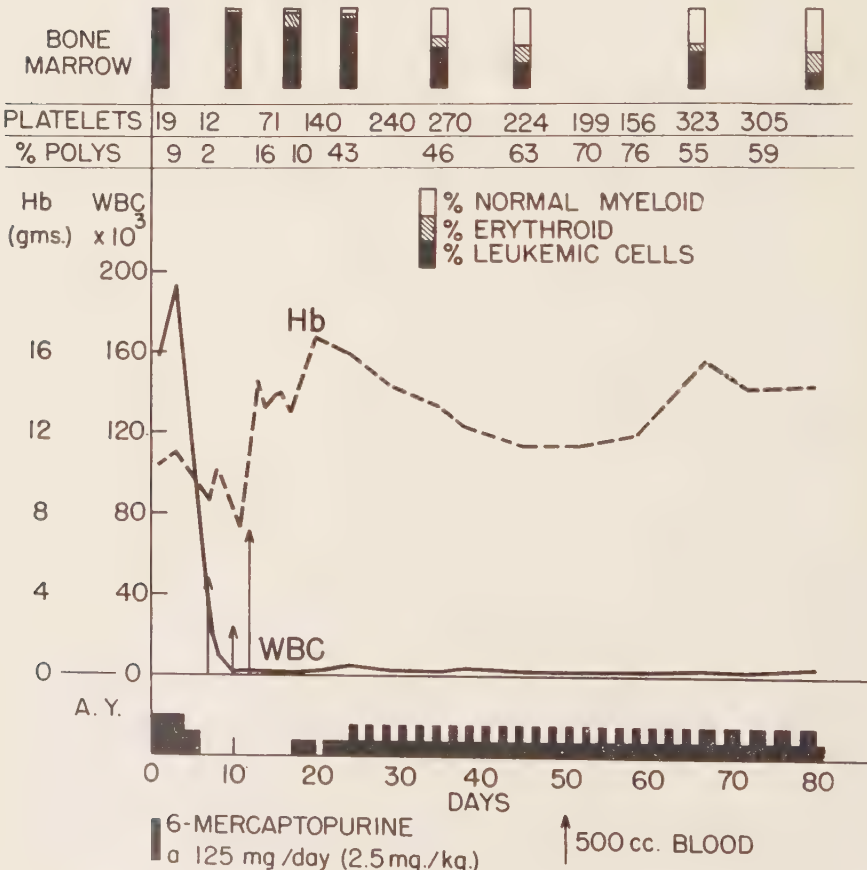


FIGURE 7

Previous preliminary studies have not shown 6-mercaptopurine to be synergistic with A-Methopterin or with cortisone in patients. Because Clarke *et al.*<sup>9</sup> demonstrated that 6-mercaptopurine and azaserine were synergistic in their inhibitory action on Sarcoma 180 and the fact that these results could be confirmed in mouse leukemia,<sup>10</sup> this combination was tried in children with acute leukemia. At the present time it is possible only to give results of preliminary studies on this combination; no definite conclusion as to its value can be reached despite the fact that this mode of therapy has been under study for the past 11 months. The cases were divided roughly into three groups.

The first group consisted of those children who were started on the combination from the beginning of their disease. In this group there were 19 patients, and among them there occurred 14 good clinical and hematological remissions, 2 partial remissions, and 3 failures. These remissions lasted from 12 to 35 weeks. This rate would appear to be a higher incidence of remissions than has generally been reported with 6-mercaptopurine alone, but it should be noted that these patients were specially selected as cases in whom it appeared that there would be adequate time for a trial of such combination therapy.

The second group consisted of 15 children whose disease was in remission on a maintenance dose of mercaptopurine and had azaserine added in an attempt to prolong the remission. The average total remission was 21 weeks, the longest lasting 34 weeks. This total would seem to represent a slight but probably not significant increase over the length of remissions achieved by mercaptopurine alone.

The third group consisted of 15 children whose disease was resistant to mercaptopurine when combination therapy was initiated. There were nine complete failures to respond to combination therapy and six very temporary remissions, lasting from 1½ to 10 weeks, the average being 4 weeks. These remissions were so short that it is questionable whether they were of much practical value. Two other patients, however, were brought into remission with a 2-week course of cortisone by mouth after one of them had become steadily worse during a 3-week course of 6-mercaptopurine alone; the other had previously responded to 6-mercaptopurine for five months and had then relapsed despite maintenance therapy up to 5 mgm./kg. daily. Upon being brought into remission by cortisone administered orally, steroid therapy was discontinued and, 2 to 4 weeks later, while the cortisone remissions were still at their height, combination therapy with 6-mercaptopurine and azaserine was started at 2.5 mgm./kg. of each drug daily by mouth. Except for an occasional reduction in the dosage of azaserine for short periods of time when sore tongue developed, both patients continued on the combination and remained in good remission for another 10-month period (FIGURE 8). Whether these two cases represent prolonged remissions from a 2-week course of cortisone, from the combination of azaserine and mercaptopurine, or from the sequential combination of all three agents, is impossible to state at the present time.

Further studies will be necessary to determine whether any practical advan-



tage accrues from the addition of azaserine to the mercaptopurine therapy. Varying the dosage of the two components of this combination should be tried, and it may be that better results can thus be achieved.

In isolated cases of acute leukemia in adults it would appear that survival time has been increased by mercaptopurine therapy, but the percentage in which such benefit occurs is so small that it is not possible to demonstrate a statistically valid increase in over-all survival time in adults having this disease.

In the treatment of acute leukemia in children, we now have at our disposal three types of agents: the folic acid antagonists, the purine antagonists, and the hormones. Fortunately, there is no cross-resistance between the different groups, and the patient whose disease has become resistant to mercaptopurine may still be expected to respond to A-Methopterin or cortisone and vice versa. All of these drugs are useful, but differences in rapidity of action, duration of remissions induced, height of the leukocyte count, and age of the patient suggest the use of a given drug at a particular time. It is our feeling that, in the treatment of the acute leukemias, the antimetabolites, A-Methopterin and mercaptopurine, should be the main reliance of the chemotherapist, with the hormones kept in reserve for emergency situations where the disease has become resistant to the antimetabolites, or where there is not sufficient time for the use of these slower acting drugs. By using the various drugs available

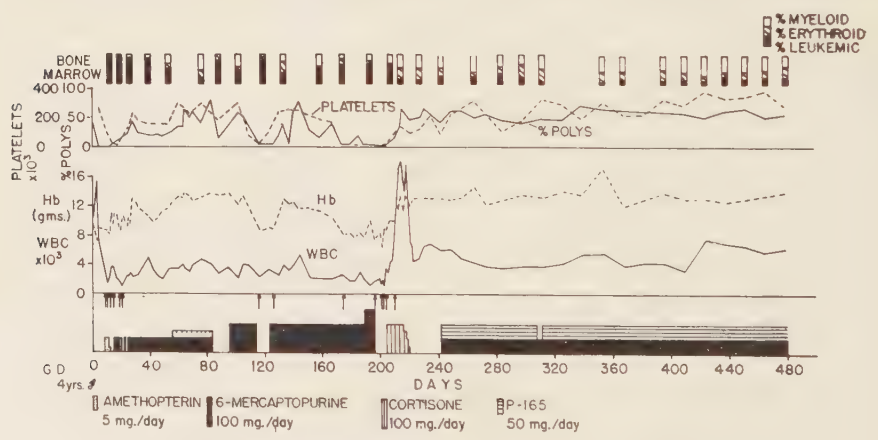


FIGURE 8

TABLE 2  
SEQUENTIAL THERAPY OF ACUTE LEUKEMIA IN CHILDREN

	Patients	Surviving 1 year or more
No treatment.....	218	5%
Folic acid antagonists and/or cortisone.....	154	29%
Folic acid antagonists, cortisone, and 6-mercaptopurine.....	52	52%

in the sequence indicated above, definite increase in survival time can be demonstrated. TABLE 2 shows the survival time of three different groups of children having acute leukemia. In 218 untreated cases collected from the literature by Tivey,<sup>11</sup> 50 per cent were dead by 3.9 months, and only 5 per cent were alive 12 months after the start of their disease. In a second series of 154 patients treated at Memorial Hospital with the folic acid antagonists and/or the steroids, 50 per cent were dead by 8.9 months and 29 per cent were living 12 months after the start of their disease. In the third group of 52 cases, whose initial treatment was begun between June 1952 and May 1953 after the time mercaptopurine became available in addition to the antifolics and the steroids, 50 per cent were dead by just over 12 months, and the 12-month survival was 52 per cent. Since the latter two series had equal amounts of antibiotics and transfusions, it would seem that the addition of mercaptopurine to a regimen employing the other two types of agents has caused a definite increase in the survival time of these children.

### Summary

(1) 6-Mercaptopurine has been studied in 269 patients having various forms of neoplastic disease.

(2) It was without practical beneficial effect in the metastatic carcinomas, sarcomas, Hodgkin's disease, lymphosarcomas, and chronic lymphocytic leukemia that have been studied.

(3) It caused occasional very temporary regressions of metastatic reticulum cell sarcoma, both subcutaneous and osseous metastases.

(4) A high percentage of remissions were achieved by using 6-mercaptopurine in the early stage of chronic myelocytic leukemia, but more time is needed to determine whether this agent will be as practical as the more conventional agents such as X ray,  $P^{32}$ , or urethane. It may be of some very temporary value in the terminal acute stage of this disease.

(5) 6-Mercaptopurine has an added advantage over some of the agents presently in use in that it will frequently cause remissions in patients resistant to A-Methopterin or cortisone, and occasionally in those having myelomonocytoid or monocytic leukemia, and in adults over the age of 40.

(6) When used in sequence with the other drugs available, it appears to cause a definite increase in the survival time of children afflicted with this disease.

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# STATISTICAL ANALYSIS OF CLINICAL RESULTS FROM 6-MERCAPTOPURINE

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New York, N. Y.*

*Preliminary remarks.* The analysis is based on the summary sheets provided by 21 participants at the conference. The summary sheet listed 13 diagnoses, but most of the data concerned acute leukemia (excepting monocytic) in children and adults (over 15 years). Except for leukemias, the data were too scanty for analysis.

The summary sheet also listed five outcomes. "Good hematologic and clinical remission" (henceforth termed "remission") was defined as one in which the peripheral blood returns to normal and the marrow has a combined total of less than 30 per cent lymphocytes and stem cells (or progranulocytes or monocytes). "Partial remission" was defined as clinical improvement with some improvement of peripheral blood and marrow but not enough to meet the standard for "remission." "Clinical remission only" was not defined on the summary sheet, and it was apparent from the summaries that some investigators did not use this category. Therefore "partial remission" and "clinical remission only" were combined into a single category henceforth termed "intermediate." The failures were divided into two categories, "under three weeks" and "over three weeks," but since several summaries did not make this distinction, it was necessary to combine the results into a single category of "failure."

The summary sheets included columns for "dosage schedule and range" and "remarks" but, unfortunately, this information could not be used in the analysis. Not only are the combination therapies and dosages quite different from one series to the next, but even within a series there was often a variety of dosage schedules. It should be clearly understood that in a large proportion of the cases listed here, 6-mercaptopurine was used in conjunction with *other therapeutic agents*.

The two largest series are those of Burchenal and Farber. Excluding monocytic, Burchenal reported on 103 children and 43 adults, and Farber reported on 55 children. To facilitate analysis a rule was made that only those series with eight or more cases of a given kind would be included in this report. In addition to the Burchenal and Farber series, there were 11 series of children (174 cases) and 9 series of adults (113 cases).

*Results with 6-mercaptopurine in children having acute leukemia (excluding monocytic).* The percentage of remissions reported vary widely from one series to the next, as is evident from TABLE I.

The per cent of remissions reported in TABLE I range from 8 per cent to 67 per cent. Most of the series are short and, therefore, subject to a great deal of sampling variation. However, there is much more variation in the per cent remissions that were reported than can be accounted for by sampling variation alone. Considering only the two largest series, Burchenal and Farber, there



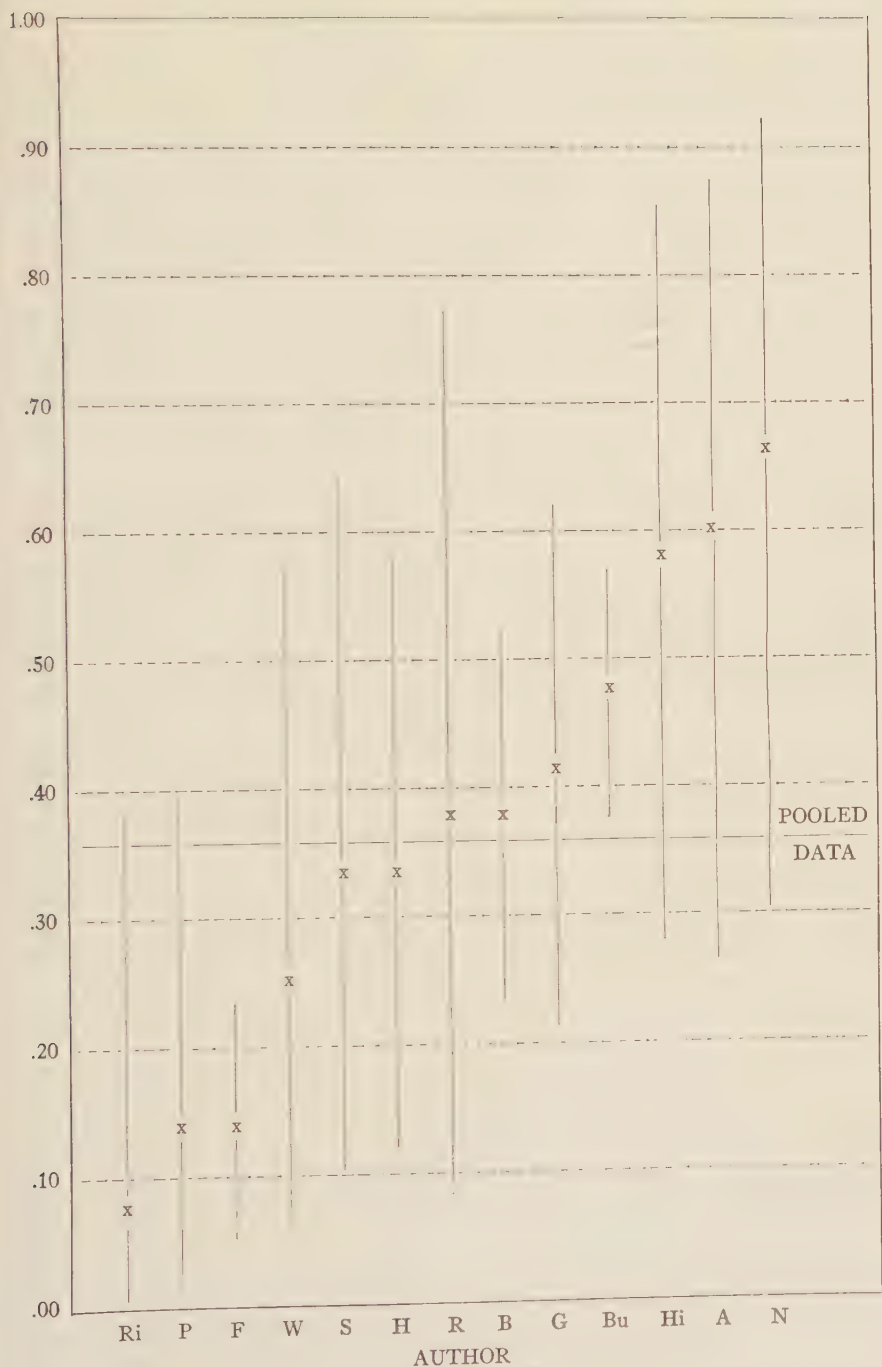
TABLE 1  
REMISSIONS: ACUTE LEUKEMIA (EXCEPTING MONOCYTIC) IN CHILDREN

Author	Number of cases	Number of remissions	Per cent of remissions	Confidence intervals	
				Lower limit	Upper limit
Rice.....	12	1	8	0.2	38
Pierce.....	15	2	13	2	40
Farber.....	55	7	13	5	24
Wilson.....	12	3	25	6	57
Sawitsky.....	12	4	33	10	65
Hyman.....	18	6	33	13	59
Rundles.....	8	3	38	8	76
Bernard.....	42	16	38	23	53
Gaffney.....	24	10	42	22	63
Burchenal.....	103	49	48	38	57
Hill.....	12	7	58	28	85
Asua.....	10	6	60	26	88
Newton.....	9	6	67	30	92
Pooled Data.....	332	120	36		

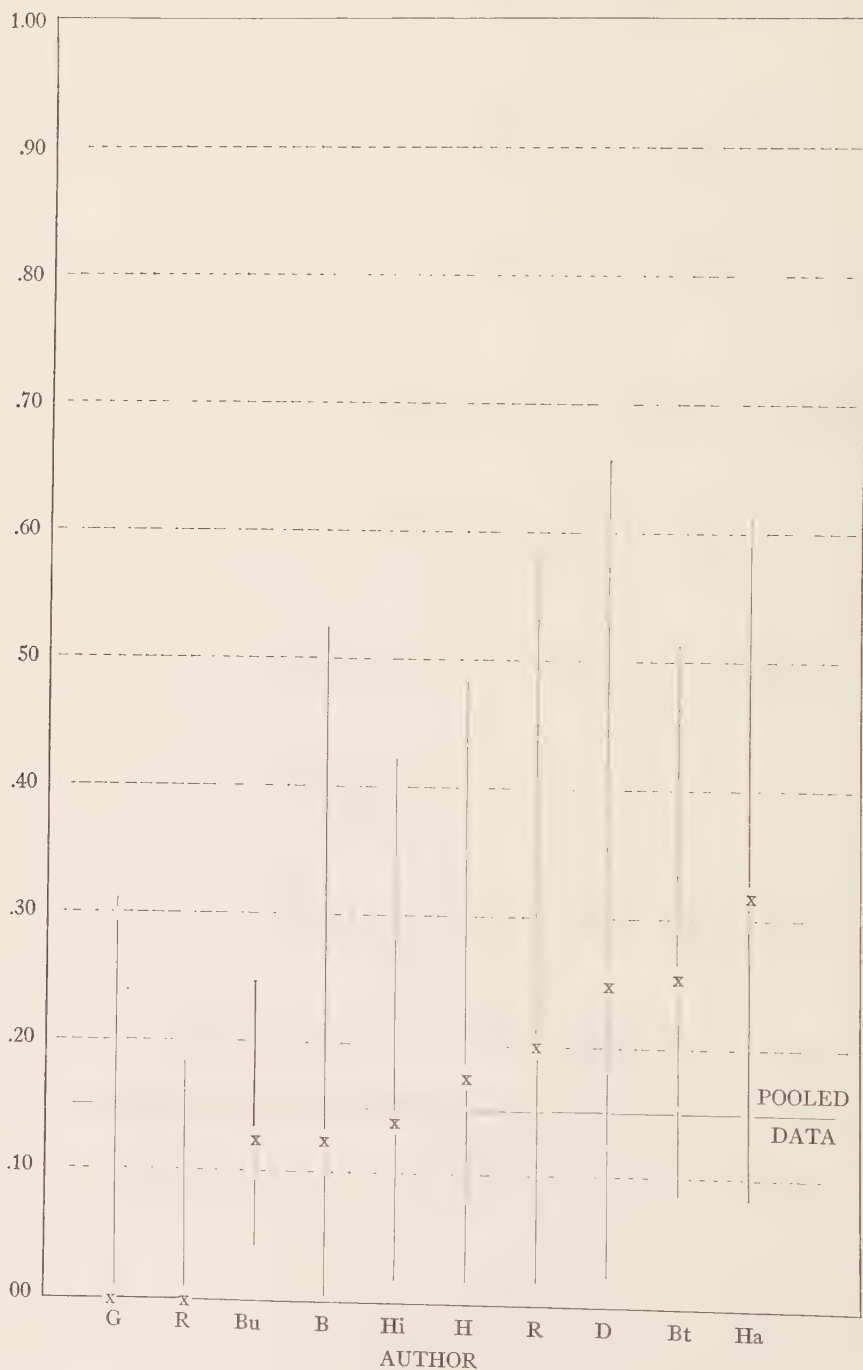
TABLE 2  
REMISSIONS: ACUTE LEUKEMIA (EXCEPTING MONOCYTIC) IN ADULTS

Author	Number of cases	Number of remissions	Per cent of remissions	Confidence Intervals	
				Lower limit	Upper limit
Gaffney.....	10	0	0	0	31
Rosenthal.....	19	0	0	0	18
Burchenal.....	43	5	12	4	25
Bernard.....	8	1	12	0.3	53
Hill.....	14	2	14	2	43
Hyman.....	12	2	17	2	48
Rundles.....	10	2	20	2	57
Demarsh.....	8	2	25	3	65
Bethell.....	19	5	26	9	51
Hall.....	13	4	31	9	62
Pooled Data.....	156	23	15		

is evidence of a marked difference in the per cent remissions (chi-square is 17.5 with one degree of freedom). A test of the remaining 11 series (taking into account intermediate results as well as remissions) also indicates significant differences (chi-square is 43 with 20 degrees of freedom). It is not difficult to suggest possible explanations for the differences (such as the use of different standards of judging remissions). In any event the significance tests provide a warning that, although the over-all per cent of remissions in these data is 36 per cent, the clinician should be cautious in using this figure as a basis of comparison for his own series. If more uniform and comprehensive reporting procedures were instituted it would be possible to obtain more concordant and



GRAPH 1  
 REPORTED PROPORTION OF REMISSIONS  
 (Acute Leukemia in Children—Excluding Monocytic)



GRAPH 2  
 REPORTED PROPORTION OF REMISSIONS  
 (Acute Leukemia in Adults—Excluding Monocytic)

reliable information on the efficacy of 6-mercaptopurine and other chemotherapies that may be developed in the future.

*Results with 6-mercaptopurine in adults having acute leukemia (excluding monocytic).* The percentage of remissions reported are more consistent in this data as is evident from TABLE 2.

The relative homogeneity of the 10 series in TABLE 2 is indicated by the fact that all of the confidence intervals include the over-all per cent of remissions, 15 per cent. The per cent remissions range from 0 per cent to 31 per cent a dispersion, which can be accounted for on the basis of sampling variation alone. If intermediate results as well as remissions are taken into account, then for the nine shorter series the value of chi-square is 19 with 16 degrees of freedom, which is not significant.

*Further Questions.* The data provides a limited amount of evidence on some other questions of interest.

- (1) Is 6-mercaptopurine less effective in children with monocytic than in children with other types of acute leukemia?

Data: All 14 reported monocytic cases and the series of 174 nonmonocytic cases.

Rank t-test: 1.8 (not significant).

Conclusions: No strong evidence of a difference between the two series.

However, the monocytic series is very short and scattered.

- (2) Is 6-mercaptopurine less effective in adults with monocytic than in adults with other types of acute leukemia?

Data: All 30 reported monocytic cases and the series of 113 nonmonocytic cases.

Rank t-test: 0.2 (not significant).

Conclusions: No evidence of a difference between the two series. However, the monocytic series is scattered.

- (3) Is 6-mercaptopurine more effective in children than adults?

Data: Burchenal series of 89 children and 49 adults.

Rank t-test: 14.1 (highly significant).

Conclusion: Children do better than adults. This inference is also strongly confirmed by the data of the other investigators.

The rank t-test is significant (.05) if it exceeds 3.84 and is very significant (.01) if it exceeds 6.64.

*Summary.* In the series considered: (1) Remissions occurred in approximately one out of three cases of children having leukemia (excluding monocytic) who were treated with 6-mercaptopurine and possibly other agents; (2) remissions occurred in about one out of seven adults having acute leukemia (excluding monocytic) who were treated with 6-mercaptopurine and possibly other agents; (3) there is no strong evidence of a difference in the proportion of remissions between the 14 reported monocytic cases and the 174 nonmonocytic cases in children; (4) there is no evidence of a difference between the 30 reported monocytic cases and the 113 nonmonocytic cases in adults; and (5) there is a highly significant difference in the proportion of remissions between children and adults.



## CLINICAL EXPERIENCE WITH 6-MERCAPTOPURINE IN HUMAN NEOPLASIA\*

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This report is concerned with the results obtained from the oral administration of 6-mercaptopurine (6-MP) in 24 adult patients and 1 child with acute leukemia, 5 patients with chronic granulocytic leukemia, 1 patient with chronic lymphocytic leukemia, 2 patients with myeloma, and 9 patients with various types of miscellaneous metastatic cancers. Of the 25 patients with acute leukemia, 10 were lymphocytic, 7 were monocytic, and 8 were granulocytic. Six of the granulocytic leukemias were acute from onset, and 2 represented the acute terminal phase of chronic granulocytic leukemia.

### *Acute Leukemia*

In assessing the results of 6-MP therapy in the acute leukemias we have defined a "complete" remission as one in which there is a disappearance of the clinical manifestations of the disease and a return of the peripheral blood and bone marrow pictures essentially to normal, the bone marrow containing 5 per cent or less of stem cells and prolymphocytes, progranulocytes, or promonocytes. These criteria are somewhat more rigid than those employed by Burchenal *et al.*,<sup>1</sup> but are utilized because our series deals largely with adults in whom, under normal conditions, the lymphocyte and stem-cell content of the bone marrow is low. A "partial" remission is one in which there is clinical and hematologic improvement, but in which restoration of essentially normal blood and bone-marrow patterns are not obtained. While our series of leukemia patients is relatively small, we have compiled data relating to survival time from date of onset of symptoms, date of diagnosis, and date of institution of 6-MP therapy in the hope that other participants in this conference will present similar data. An analysis of composite data then can be made and compared with the results of other methods of treatment.

*Administration, dosage, and method of procedure.* As a rule, 6-MP was administered in a single oral dose daily, but in a few instances in which anorexia and nausea developed, the drug was administered in two equally divided doses daily. Dosage schedules varied from 1.5 to 6.7 mgm./kg. of body weight each day. Because Clarke and his associates<sup>2</sup> had demonstrated the development of resistance to 6-MP in tumor cells, higher doses of the drug often were administered during the early stage of treatment. Subsequently, when responses had been observed, lower doses were employed. During periods of treatment, blood examinations were made two to three times weekly, and bone-marrow aspirations were performed every week or every second week. Changes in the bone marrow were found to be a more reliable therapeutic guide than the periph-

\* Contribution of the Cooperative Cancer Chemotherapy Research Program of the National Institutes of Health, U. S. Public Health Service.

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eral blood picture. Whenever possible, treatment with 6-MP was carried to the point of producing marked hypoplasia of the bone marrow. In such instances, leukemic leukocytes (stem cells, prolymphocytes, progranulocytes, or promonocytes) disappeared before complete suppression of erythropoietic elements took place. Not infrequently, severe degrees of leukopenia (less than 1000 leukocytes per cm.) were observed for days or even weeks before the leukemic leukocytes disappeared from the marrow. Transfusions of blood were administered at intervals during treatment, but antibiotic preparations were not given unless evidence of infection was observed. Following the disappearance of leukemic leukocytes from the bone marrow, the administration of 6-MP was discontinued and the patient was supported with blood transfusions, spaced at intervals to maintain a hemoglobin level of 7.5 to 8.0 gm./100 cc. of whole blood. In a number of instances following cessation of 6-MP therapy, normal hematopoiesis was restored within two to three weeks after cessation of therapy. In other instances, the regrowth of normal hematopoietic elements was accompanied by the simultaneous regrowth of leukemic leukocytes. Nevertheless, in the latter, good clinical and partial hematologic remissions usually were observed.

In most instances, when essentially complete clinical and hematologic remission was obtained, 6-MP therapy was not resumed until evidence of relapse occurred. However, in all patients showing a partial but incomplete response, 6-MP was administered continuously or intermittently for comparatively long periods of time.

*Toxicity.* Toxic manifestations are listed in TABLE 1. We have not included signs of suppression of bone-marrow activity (leukopenia, anemia, thrombocytopenia) among the manifestations of toxicity, because we feel they are an essential part of the therapeutic procedure. Anorexia, nausea, and vomiting were observed most frequently among patients receiving comparatively large doses of 6-MP (4.0 mgm./kg. or more). They were not as common among patients receiving 3.0 mgm./kg. or less. Stomatitis was encountered in approximately one fourth of our cases, and was characterized by the development of exudative or ulcerative lesions on the mucous membranes of the oral cavity. Whenever lesions of this type were observed, treatment was discontinued for a

TABLE 1  
TOXIC MANIFESTATIONS OF 6-MP IN 25 PATIENTS WITH ACUTE LEUKEMIA

Manifestation	No. of Patients	Incidence, per cent
Anorexia.....	8	32
Nausea.....	7	28
Stomatitis.....	6	24
Vomiting.....	4	16
Diarrhea.....	2	8
Proctitis.....	1	4
Fever.....	1	4
Rhinitis.....	1	4
Swollen tongue.....	1	4
Melena.....	1	4

few days until the lesions healed, then the administration of 6-MP was resumed in lower dosage. The development of either diarrhea or melena, which could result from ulceration of the lower gastrointestinal tract, also was considered an indication for temporary cessation of 6-MP therapy.

*Results.* Details relating to dosage schedules, duration of therapy, incidence and duration of remission, *etc.*, are given in TABLE 2. In addition, the current status of the patient and the survival time from onset of symptoms and from date of diagnosis are listed.

An initial remission rate of 56 per cent was noted for the entire group of 25 cases of acute leukemia (or 54 per cent for the 24 adults having the acute form of the disease), a figure somewhat lower than that previously reported by us for a smaller series of cases.<sup>3</sup> Nevertheless, this rate of response to 6-MP therapy is considerably higher than that obtained in adults with acute leukemia treated with the folic acid antagonists or with hormones.

Review of the data in TABLE 2 has shown that, in adult patients, partial remission seldom is obtained with total doses of 6-MP amounting to less than 2250 mgm., and complete remission with doses less than 3900 mgm. If 2250 mgm. is accepted as the minimal amount of 6-MP required to induce some degree of remission, then treatment was inadequate in 6 patients in our series (cases 16, 20 to 25), and ineffectual in 7 patients (cases 8, 9, 10, 13 to 15, 17). The incidence of initial remission was found to be higher, and the duration of remission was longer among patients having acute lymphocytic leukemia than among those having acute monocytic or acute granulocytic leukemia (TABLE 3).

An analysis of five patients with acute leukemia living six months or more after institution of 6-MP therapy is listed in Table 4. One patient with acute aleukemic lymphocytic leukemia and one with acute monocytic leukemia (cases 4 and 11, TABLE 2) are in essentially complete remission 7<sup>1</sup>/<sub>2</sub> and 7 months respectively after cessation of treatment. One patient (case 2, TABLE 2), in whom a complete clinical and hematologic remission of two months duration was obtained after three relatively short courses of therapy administered over a period of 11 weeks, subsequently relapsed, and currently is being treated with 6-MP and azaserine (O-diazoacetyl-L-serine). The remaining two patients are on maintenance therapy with 6-MP.

The induction of more than one remission has been observed in a number of instances (TABLE 2). As a rule, the second remission has been shorter in duration than the first, and larger amounts of 6-MP are required to control the disease. The development of resistance to 6-MP is difficult to assess, but we have gained the impression that this phenomenon does occur on a program of intermittent therapy. Therefore, despite the observation of remission of several months duration after cessation of treatment in two patients, we are inclined to favor a program of continued administration of the drug.

### *Chronic Leukemia*

The results of 6-MP therapy in chronic leukemia are shown in TABLE 5. Excellent clinical and hematologic responses were observed in five patients having chronic granulocytic leukemia. In general, the drug was tolerated

well, but in one instance (case 28), treatment was discontinued because of persistent nausea and anorexia. Following induction of remission and cessation of therapy, relapse occurred in every case in from three to six weeks. Maintenance therapeutic programs have accordingly been instituted for the four patients tolerating this form of therapy. No clinical or hematologic improvement was noted in one patient with chronic lymphocytic leukemia after three weeks of treatment with 6-MP, an observation in accord with the report of Burchenal *et al.*<sup>1</sup>

### *Myeloma*

6-MP was administered to two patients with myeloma for four and three weeks respectively (TABLE 6). While the periods of treatment were relatively short, no symptomatic or objective evidence of improvement was observed.

### *Miscellaneous Cancers*

Nine patients with various types of metastatic cancer were treated with 6-MP. The results are tabulated in TABLE 7. There was no evidence of improvement among three patients having bronchogenic carcinoma, one patient with retroperitoneal sarcoma, one patient with squamous cell carcinoma of the esophagus, one patient with lympho-epithelioma of the pharynx, and one patient with anaplastic carcinoma. One patient with Ewing's tumor (case 42) has shown no roentgenologic evidence of progression of bone lesions after 26 weeks of treatment with 6-MP. During this period he has been free of bone pain. Another patient (case 41) with anaplastic carcinoma (primary site unknown) showed remarkable regression of metastatic lesions involving the skin, lymph nodes, and lungs on 6-MP therapy. He remained in reasonably good health on continuous administration of the drug for a period of six weeks despite an extreme degree of leukopenia (225 to 600 leukocytes per cu. mm.) and thrombocytopenia (less than 20,000 platelets per cu. mm.). Owing to a misunderstanding attributable to language difficulties, he discontinued taking the drug and died five days later, following rapid growth of original and new metastatic lesions.

### *Discussion*

On the basis of the data presented in this and other reports,<sup>1, 3</sup> the anti-metabolite, 6-mercaptopurine, appears to be a valuable addition to the list of chemotherapeutic agents currently available for the treatment of certain types of neoplastic disease. It has proved to be decidedly more efficacious than adrenocorticotrophic hormone (ACTH), cortisone, or the folic acid antagonists in the treatment of adult patients with acute leukemia. In children with acute leukemia, the results of 6-mercaptopurine therapy are comparable to, but probably not better, than those obtained with the folic acid antagonists.<sup>1</sup> In chronic granulocytic leukemia, favorable clinical and hematologic responses may be obtained from the administration of 6-mercaptopurine, but continued administration of the compound is required to prevent relapse. In the treatment of chronic lymphocytic leukemia, myeloma, and metastatic "solid" tumors, 6-



TABLE 2  
RESULTS OF 6-MP THERAPY IN ACUTE LEUKEMIA

Case #	Diagnosis	Age Sex	Previous Rx	Administration of 6-MP			Duration of Rx. days	Remission mo.	Date of onset of symps.	Date of diag.	S.T. from onset of symps. mo.	S.T. from date of diag. mo.	Present Status
				No. of Courses	Daily dose mgm./kg.	Total dose mg.							
1	A.L.L.	M 39	None	1	2.3	5775	34	Partial, 2	Dec. '52	6/1/53	16	9 $\frac{1}{4}$	Living & well on maintenance therapy 9 mos. after starting Rx.
				2	4.6	7100	29	" 2					
				3	2.0-2.5	7900	62	" $\frac{1}{2}$					
				4	2.0-2.5	5400	36						
2	A.L.L.	F 45	Spray X-ray ACTH	1	3.1-6.1	2250	10	Partial, 1 $\frac{1}{2}$	Mar. '53	Jun. '53	13	10	Living but in relapse 6 $\frac{1}{2}$ mos. after starting Rx. Under Rx. 6-MP & Azaserine at present
				2	6.1	3750	14	" $\frac{1}{2}$					
				3	1.5	1300	13	Complete, 2					
				4	Azaserine & 6-MP			—					
3	Ac.A.L.L.	F 15	None	1	2.5	4000	39	Complete, 2	Aug. '53	9/24/53	7	6 $\frac{1}{2}$	Living; in relapse 6 $\frac{1}{2}$ mo. after starting Rx. Still under Rx.
				2		5050	37	Partial, 1					
				3		750	5	None					
				4		1000	10	—					
4	Ac.A.L.L.	F 35	Cortisone 2 $\frac{1}{2}$ mo. remission	1	2.5	6900	46	Complete, 6 mo.	Dec. '52	Jun. '53	16	9 $\frac{1}{4}$	Living & well 7 $\frac{1}{2}$ mos. after starting Rx.
5	A.L.L.	M 28	None	1	2.5-4.2	2650	17	Partial	Jan. '54	2/15/54	3 $\frac{1}{4}$	1 $\frac{3}{4}$	Living & well 1 $\frac{1}{2}$ mo. after starting Rx.
				2		2125	16	Partial					
6	Ac.A.L.L.	F 6	None	1	2.7-5.5	1400	27	Partial	Feb. '54	3/10/54	2 $\frac{1}{4}$	1	Living & well 1 mo. after starting Rx.
7	A.L.L.	F 29	Cortisone 3 $\frac{1}{2}$ mo. remission	1	2.8	4750	32	Partial, 1	Mar. '52	Mar. '53	19	7	Died 2 $\frac{3}{4}$ mos. after starting 6-MP Rx.
				2	6.0	9900	33	Transient					

TABLE 2 (continued)

8	S.A.L.L.	M 61	TEM	1	2.8-5.0	5050	17	None	Jul. '52	Sep. '52	18½	16½	Died 3½ mo. after starting 6-MP Rx.
9	Ac.A.L.L.	F 48	None	1	3.2	3150	17	None	Feb. '53	9/22/53	9¾	1	Died 3 wks. after starting 6-MP Rx.
10	A.L.L.	M 29	None	1	3.0	2600	13	None	Sep. '53	10/29/53	2¾	1	Died 13 days after starting 6-MP Rx.
11	A.M.L.	M 44	None	1	2.3	3900	26	Complete, 6	May '53	9/17/53	11	7	Living & well 7 mo. after starting Rx.
12	A.M.L.	F 33	Cortisone	1	3.4	3200	15	Partial, ½	Jan. '54	2/28/54	3¼	1¼	Markedly improved 2 wks. after starting Rx.
13	A.M.L.	M 54	Cortisone, Myeleran	1 2	4.1-6.7 1.4-2.8	9250 2650	24 22	Partial, 1½ None	Apr. '53	4/9/53	9½	9	Died 3½ mo. after starting 6-MP Rx.
14	A.M.L.	M 33	None	1	2.5-3.0	5850	34	Partial, 1½	Jul. '53	10/12/53	6	3	Died 3 mo. after starting Rx.
15	A.M.L.	M 78	None	1	3.0	2400	12	None	Jul. '53	7/16/53	3½	3	Died 14 days after starting Rx.
16	A.M.L.	M 31	Myeleran	1	4.5	1800	6	None	May '53	Jun. '53	5½	4½	Died 6 days after starting Rx.
17	A.M.L.	M 33	None	1	4.2	3500	14	None	Dec. '53	1/15/54	2	1	Died 1 mo. after starting Rx.
18	A.G.L.	F 31	None	1 2 3 4	2.8 2.4-3.8 2.4 Azaserine + 6-MP	1800 2400 1500	12 18 12 9	Partial, ½ None None Partial	Sep. '53	2/17/53	7¼	3¾	Living & in partial remission 3¾ mo. after starting Rx.

TABLE 2 (continued)

Case #	Diagnosis	Age Sex	Previous Rx	Administration of 6-MP			Duration of Rx, days	Remission mo.	Date of onset of symps.	Date of diag.	S.T. from onset of symps. mo.	S.T. from date of diag. mo.	Present Status
				No. of Courses	Daily dose mgm./kg	Total dose mg.							
19	A.G.L.	F 18	None	1 2	2.7-3.4 Unknown	6850 under treatment elsewhere	32	Partial, 1½	Nov. '53	12/28/53	5½	3½	Living but in relapse 3½ mo. after starting Rx.
20	A.G.L.	M 73	None	1	2.6-3.5	1450	9	None	Jan. '53	Mar. '53	8¾	5¾	Died 10 days after starting Rx.
21	A.G.L.	F 73	None	1	2.5	1800	12	None	Oct. '53	11/24/53	2½	1¼	Acute psychosis. Died 3 wks. after starting Rx.
22	A.G.L.	F 65	None	1	3.9	1750	7	None	Feb. '54	3/25/54	2¼	<½	Cerebral hemorrhage. Died 13 days after starting Rx.
23	A.G.L.	F 51	None	1	3.1	600	3	None	3/22/54 mild CVA	3/25/54	1½	<¼	Cerebral hemorrhage. Died 4 days after starting Rx.
24	Ac. phase of C.G.L.	F 48	Myeleran	1	2.5 5.0	2250	11	Partial, 1	1945	1945	106½	106½	Died 6 wks. after starting 6-MP Rx.
25	Ac. phase of C.G.L.	M 53	Total body irradiation P-32	1	3.5	550	2	None	Jan. '50	Jan. '50	47½	47½	Died 2 days after starting 6-MP Rx.

TABLE 3

INCIDENCE OF INITIAL REMISSION WITH 6-MP THERAPY IN ACUTE LEUKEMIA

Type of Leukemia	Total No. Treated	Remission rate		Duration of remission after Rx. mo.
		Number	Per cent	
Acute lymphocytic	10	7	70	2-7½ (2 cases still under Rx.)
Acute monocytic	7	4	57	½-6 (1 case still under Rx.)
Acute granulocytic	8	3	37.5	½-1½ (2 cases still under Rx.)
Average remission rate	25	14	56	

TABLE 4

ANALYSIS OF ACUTE LEUKEMIA CASES LIVING SIX MONTHS OR MORE AFTER INSTITUTION OF 6-MP THERAPY

Case #	Diagnosis	Sex, Age	Duration months	Total amount given, mgm.	Current status
1	A.L.L.	M., 39	9	25,525	On maintenance Rx.
2	A.L.L.	F., 45	6½	14,500	On 6-M.P. & Aza-serine
3	A.A.L.L.L.	F., 15	6½	10,800	On maintenance Rx.
4	A.A.L.L.L.	F., 35	7½	6,900	No Rx. since 9/12/53
5	A.M.L.	M., 44	7	3,900	No Rx. since 10/11/53

mercaptopurine is ineffective although transient regression of metastasis was noted in one patient with anaplastic carcinoma.

In adult patients with acute leukemia, it is possible to induce essentially complete remission by administering 6-mercaptopurine in a dosage of from 2.5 to 6.0 mgm./kg. of body weight for a period sufficient to eradicate leukemic leukocytes from the bone marrow. To accomplish this result, the drug is given continuously, despite marked suppression of hemopoietic activity, until "stem" cells, prolymphocytes, promonocytes, and/or progranulocytes virtually disappear from the bone marrow. Serial bone-marrow aspirations therefore must be performed, the marrow picture rather than the peripheral blood being used as the primary therapeutic guide. Therapy is interrupted temporarily if exudative or ulcerative lesions involving the mucous membranes of the mouth or throat, or symptoms (*e.g.* melena, diarrhea, *etc.*) indicative of ulceration elsewhere in the gastrointestinal tract are observed, or if acute infection (bacteremia, abscess formation, *etc.*) develops. Infections of serious degree have been an infrequent complication despite long-standing drug-induced leukopenia. When they are encountered, an attempt is made to isolate and culture the invading organism, so that the appropriate antibiotic can be given to the patient.

The successful induction of an essentially complete remission in an occasional



TABLE 5  
RESULTS OF 6-MP THERAPY IN CHRONIC LEUKEMIA

Case #	Diagnosis	Age Sex	Previous Rx.	Administration of 6-MP			Duration of Rx, days	Response		Remarks
				No. of courses	Daily dose mgm./kg.	Total dose mgm.		Clinical	Hematologic	
26	C.G.L.	M 48	None	1	3.2-6.1	6300	26	Marked	Excellent	5 week remission.
				2	6.0	6900	23	"	"	6 "
				3	6.0	4800	16	"	"	4 "
				4	6.0	5300	20	"	"	On maintenance Rx.
27	C.G.L.	M 23	None	1	1.8-3.7	8400	32	"	"	3 wk. remission
				2	1.2-3.7	10,500	46	"	"	On maintenance Rx.
28	C.G.L.	M 52	TEM	1	2.5	2000	16	"	"	4 wk. remission; 6-MP discontinued because of nausea. Now on Myelaran.
				2	2.0	625	5	None	None	
29	C.G.L.	M 30	None	1	1.8-3.7	7650	33	Marked	Excellent	On maintenance Rx.
30	C.G.L.	F 26	None	1	1.5-3.0	3700	19	"	"	" "
31	C.L.L.	M 63	Urethane	1	3.1	7250	21	None	None	No remission.

TABLE 6  
RESULTS OF 6-MP THERAPY IN MYELOMA

Case #	Sex, Age	Administration of 6-MP		Duration of Rx., days	Response
		Daily dose mgm./kg.	Total dose mgm.		
32	M., 55	2.5	4350	29	None
33	M., 74	2.0	3150	21	None

TABLE 7  
RESULTS OF 6-MP THERAPY IN MISCELLANEOUS CANCERS

Case #	Diagnosis	Sex, age	Administration of 6-MP		Duration of Rx., days	Response
			Daily dose, mgm./kg.	Total dose mgm.		
34	Retroperitoneal sarcoma	Male 42	5.0	4200	14	None
35	Bronchogenic carcinoma	Male 62	2.4-5.0	4475	13	None
36	Bronchogenic carcinoma	Male 46	2.2-5.0	8270	37	None
37	Bronchogenic carcinoma	Male 49	2.7	4200	21	None
38	Squamous cell ca esophagus	Male 55	5.0	2200	11	None
39	Lymphoepithelioma nasopharynx	Male 24	1.5-5.0	2600	18	None
40	Anaplastic carcinoma	Male 66	4.2	6750	27	None
41	Anaplastic carcinoma	Male 55	2.1-5.7	13,550	41	Marked regression of cutaneous, lymph node, & pulmonary metastasis. Died 5 days after stopping Rx.
42	Ewing's tumor	Male 17	2.9	36,400	182	No progression of bone lesions, no bone pain

adult patient with acute leukemia by the method described above is dramatic, but it is important to emphasize that many patients having acute leukemia respond poorly, or not at all, to the administration of 6-mercaptopurine. In some, it is possible, by the continuous administration of the compound to maintain clinical, but not hematologic, remission.

#### *Summary and Conclusions*

(1) Twenty-four adult patients and one child with acute leukemia were treated with the purine antimetabolite, 6-mercaptopurine.

(2) An initial remission rate of 56 per cent for the entire group, or 54 per cent for the adult patients, was obtained.

(3) The rate of initial remission for the adult patients with acute leukemia reported in this communication is significantly higher than that noted previously with folic acid antagonist or hormone therapy.

(4) Remissions may be partial or essentially complete. In order to induce an essentially complete clinical and hematologic remission, it is necessary to administer 6-mercaptopurine to the point that production of leukemic leukocytes in the bone marrow is completely or almost completely suppressed. Suppression of this degree is associated with suppression of normal hematopoiesis, but following withdrawal of the drug, regeneration of the normal constituents of the bone marrow takes place within a few days.

(5) Essentially complete remission may last for several months and does not require the continued administration of 6-mercaptopurine. When relapse occurs, a second remission sometimes can be obtained by resuming 6-mercaptopurine therapy, but the second remission usually is less complete than the first. This observation suggests that leukemic leukocytes ultimately develop a resistance to 6-mercaptopurine, possibly through the utilization of metabolic pathways that are not blocked by this antimetabolite.

(6) Control of patients with acute leukemia in partial remission is maintained most satisfactorily by the continued administration of 6-mercaptopurine.

(7) Five patients with chronic granulocytic leukemia responded well initially to the oral administration of 6-mercaptopurine, and four of these have been maintained in satisfactory clinical and hematologic remission by the continued administration of the compound.

(8) In two patients with myeloma and eight of nine patients with various types of metastatic cancers, 6-mercaptopurine therapy was of no value. Transient regression of cutaneous, lymph node, and pulmonary metastasis were observed in one patient with anaplastic carcinoma.

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# A STUDY OF 61 LEUKEMIAS TREATED WITH 6-MERCAPTOPURINE

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Since February 1953, we have treated 61 leukemias with 6-mercaptopurine (6-MP). This paper describes the results of this therapy.

*Posology and direction for treatment.* 6-Mercaptopurine was given to our patients orally, the average dose consisting of 2.5 mgm. per kg. of weight a day (for instance, a child weighing 20 kg. would take 50 mgm. a day). In some cases, when there was a relapse during a continuous treatment, we could make the daily dose 4 or 5 mgm. kg. without any noticeable disadvantage.

The length of treatment varies. It is generally long. As a rule, we continue it for nine weeks before acknowledging its failure. In cases where a good remission had occurred, we continued this treatment for six or seven months.

In most cases 6-MP was used alone. In all cases in which anemia was serious, our therapeutic plan was as follows: (1) blood transfusions every day or every other day; (2) when the number of red cells reached 4,000,000, no more transfusions, a complete blood and bone marrow study, and beginning of the treatment with 6-MP.

One advantage of this method is to avoid, during treatment with 6-MP, transfusions made necessary by severe anemia and the slow action of the drug. Another advantage is that it makes interpretation of the results easier. Further, it is hoped, by means of this method, to diminish the number of toxic accidents in therapy. Finally, the method is based primarily on the fact that the action of antileukemic medications is stronger when anemia has been cured first.

In some cases, we studied the effect of double therapeutic associations: 6-MP-cortisone, 6-MP-antifolics, or triple ones: 6-MP-cortisone-antifolics.

All the patients treated were submitted to regular clinical inspection and blood control, consisting of three blood tests a week, and a weekly test of the bone marrow.

Using 6-MP, we treated 61 patients, including 50 cases of acute leukemia; eight cases (seven adults and one child) of chronic myeloid leukemia in myeloblastic transformation; and three cases of chronic myeloid leukemia. The 50 cases of acute leukemia included 29 children and five adults not previously treated, and 13 children and three adults whose cases were resistant to other therapies.

*Incidents and accidents of the treatment.* On the whole, 6-MP has been well tolerated. We observed no anemia, nor any disorder of the red cells attributable to the treatment. In one case only there occurred thrombopenia accompanied by hemorrhages, but we must add that in this case the leukemia had reached an advanced stage, and it was not established that the drug was responsible for this development.

Leukopenia and neutropenia are more frequent. In two cases, there were



TABLE 1  
GENERAL MANAGEMENT

- (1) *Blood Transfusions Alone*  
until red-cell count reaches 4,000,000/cu. mm.
- (2) *Transfusions are Interrupted and 6-MP is Given Alone*  
Average daily dosage: 2.5 mgm./kg. to 5 mgm./kg.  
Duration: at least 9 weeks, sometimes extended to 30 weeks.

TABLE 2

Acute leukemias.....	{children adults	42 8	} 50
Myeloid chronic leukemias on acute myeloblastic transformation . .	{children adults	1 7	
Myeloid chronic leukemias.....		3	3
			61

TABLE 3  
INCIDENTS AND ACCIDENTS IN THE COURSE OF TREATMENT OF LEUKEMIAS WITH 6-MP

Cases	Anemia and erythroblastopenia	Thrombocytopenia and hemorrhage	Severe leucopenia without clinical disorders	Clinical disorders depending upon leucopenia	Various disorders
61	0	1 (?)	6	0	0

previous necrotic lesions of the mouth. However, the treatment was continued, and these necrotic lesions healed. They were probably related to the leukemia rather than to the drug. In all other cases, leucopenia was latent. Its evolution seemed to us to be rather particular. First, there was a diminution in the number of leukocytes, in the number of polymorphonuclear neutrophiles of the blood, and in the amount of young granulocytes of the marrow which had remained low. Then, as the treatment with 6-MP continued, the number of leukocytes of the blood and the number of neutrophiles rose and approximated the average number. The leukoneutropenia of the first period can be very serious. In one of the cases we observed (case No. 30), considering how serious the situation was, and how inefficacious all other therapies, we continued to use 6-MP, in spite of a very long number of leukocytes (200 per cu. mm.), and the patient experienced complete remission with a normal number of leukocytes. In this case, it is still difficult to understand the reason for this occurrence of leukopenia or to tell precisely what was the part played by leukemia, on one hand, and by the drug, on the other, but it is both important and useful to note: (1) that 6-MP is well tolerated in the conditions under which we administered it; that it can be given for leukopenic leukemia, and for leukemia with thrombopenia and hemorrhages without unfavorable effect; and (2) that the decrease in the number of leukocytes which occurs during the treatment should not be considered too dangerous, as it does not adversely affect the therapy being applied.

Table 1 sums up these observations. The occurrence of incidents and

accidents in our treatments does not seem to have been as frequent as in the treatments reported by other observers. This improvement may be attributable to the transfusions which, in the case of our patients, systematically preceded treatment with 6-MP.

### Results

*Notes.* (1) As the action of 6-MP is often very slow, we consider as valid only the observations made when treatment could be continued for at least 20 days.

(2) The differences sometimes found between statistical results are, in a great measure, attributable to lack of exactitude in defining remissions. Some optimistic observers call vague clinical improvements a remission. Our criteria are:

*Complete remission:* a normal clinical condition; a normal blood picture; and a normal myelogram, with no more than 7 per cent leucoblasts and suspect lymphoid cells.

*Satisfactory remission:* a normal clinical condition; a normal blood picture; and a myelogram containing from 7 per cent to 25 per cent leucoblasts and suspect lymphoid cells.

*Incomplete remission:* a satisfactory clinical condition; a definite blood improvement (under 1 per cent leucoblasts, and the number of polymorphonuclear neutrophiles normal again); and improvement of the marrow (25 to 50 per cent leucoblasts).

*Improvements:* in blood and clinical condition, or only in clinical condition.

*Failures:* no noticeable change.

(3) It may be useful to repeat that blood transfusions alone may cause important changes. For instance, in our case No. 5 it was noticed, through a complete examination just before 6-MP was given, that, under the influence of 10 transfusions, the amount of marrow leucoblasts had fallen from 95 per cent to 4 per cent. This eventual difficulty of interpretation caused us to avoid, as far as possible, the simultaneous association of transfusions and 6-MP.

### Acute Leukemia of Children

We have treated 42 cases of acute leukemia of children, including 29 cases not previously treated, and 13 cases resistant to other therapies.

*Acute leukemia not previously treated.* The total results are: number of cases, 29; nonvalid observations, 6; valid observations, 23; complete remissions, 8; satisfactory remissions, 4; incomplete remissions, 3; improvements, 5; failures, 3.

*Nonvalid observations.* In five cases, the action of the treatment cannot be validly appreciated, as death occurred rapidly after the treatment was initiated: on the third day, cases Nos. 25 and 26; on the fifth day, case No. 24; on the eighth day, case No. 27; on the eleventh day, case No. 28 (evolution of intercurrent measles). In a sixth case (case No. 29), the treatment was discontinued on the 15th day.

*Complete remissions.* We observed six cases of complete remissions. In these six cases, 6-MP was the only medication used. Transfusions had been administered before treatment was started.

TABLE 4  
GLOBAL RESULTS CONCERNING THERAPY WITH 6-MP OF ACUTE LEUKEMIAS IN CHILDREN

	Cases	Results not valuable	Results valuable	Complete remission	Satisfactory remission	Incomplete remission	Clinical improvement	Failure
Not treated previously.....	29	6	23	8	4	3	5	3
Resistant to other therapies.....	13	2	11	3	1	0	1	6
Total.....	42	8	34	11	5	3	6	9
				16				

This type of remission (and this feature is most remarkable) comes late. A long time elapses between the beginning of the treatment and appearance of the remission. This lapse of time was about one month (28 to 35 days) in five cases (Nos. 3, 4, 5, 7, and 8); 42 days in case No. 6; two months in case No. 2; and three months in case No. 1.

Three of these eight patients had a leucopenic form of the disease when the treatment was started.

The most schematic case to follow the evolution of hematologic disorder seems to have been case No. 1. An anemia of about 3,500,000 and a leukopenia varying from 2,000 to 3,000 continued for several weeks of the treatment.

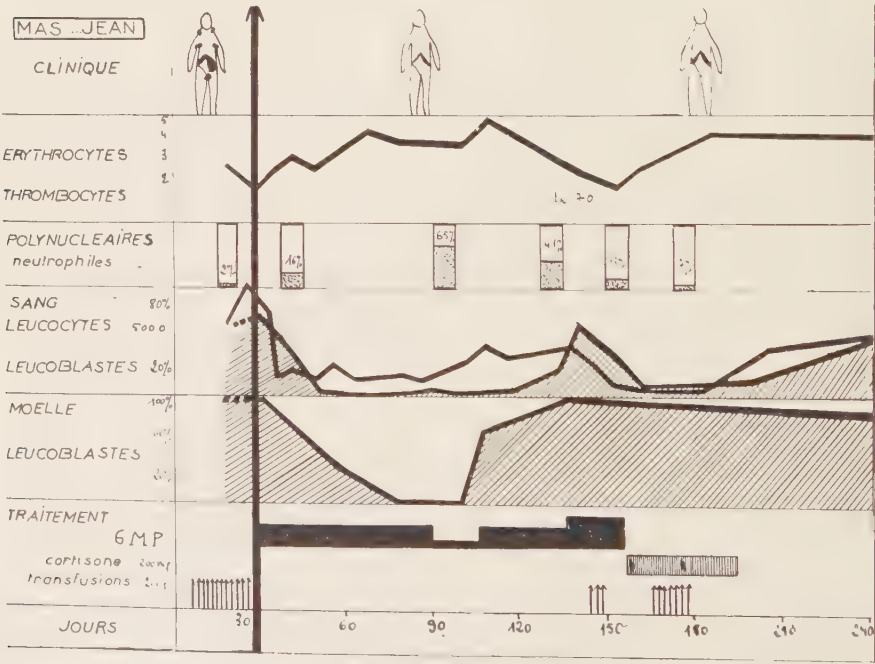


FIGURE 1





was a remission both of blood and of clinical condition after one month's treatment. Only one month later there were only 10 per cent of leucoblasts in the marrow.

In the case of these four patients, the remission was satisfactory. The amount of marrow leucoblasts decreased to 9 per cent in case No. 10; 10 per cent in case No. 9; 20 per cent in case No. 11; and 15 per cent in case No. 12 respectively.

Relapses occurred during the 6-MP treatment after a delay of 45 days in case No. 11, and after a delay of two months in case No. 9. In the latter case, cortisone and Aminopterin had good effect. In case No. 10, death was caused by septicemia and not by a relapse. The remission of case No. 12 continues.

*Incomplete remissions.* We have observed three incomplete remissions in cases Nos. 13, 14, and 15 (clinical and blood remissions almost complete). There was a decrease in marrow leucoblastosis from 95 per cent to 45 per cent in the first case; from 100 per cent to 40 per cent in the second case; and from 75 per cent to 40 per cent in the third case.

*Improvements.* The five cases in this class concern three clinical and blood improvements and two clinical improvements showing no valid blood modification. In one child, the blood leucoblastosis disappeared, but leucopenia persisted. In another, on the contrary, the number of white cells increased after four weeks' treatment. But in both cases modifications of the marrow leucoblastosis were very slight. In case No. 18 the treatment with 6-MP was con-

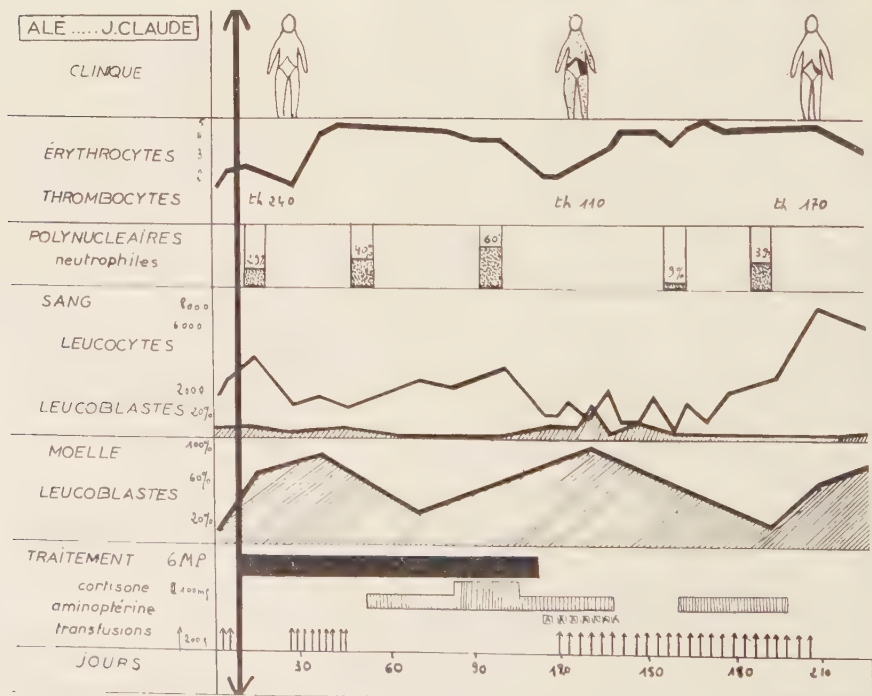


FIGURE 3

tinued for eight weeks. Later the leukemia finally proved resistant to other therapies and to 6-MP-cortisone.

In the third of these patients (case No. 19) a definite remission in blood and in clinical condition was constant but, for reasons beyond our control, there was no check of the marrow. Accordingly, in order to be on the safe side, we prefer to place it in this class.

There were two clinical improvements without any valid blood modification (cases Nos. 17 and 20).

*Failures.* Of our failures, one (case No. 23) is particularly instructive, for, during the seven weeks' treatment with 6-MP, we could observe the increasing amount of marrow leucoblasts, an amount not very high at first. Administration of cortisone and then the use of Aminopterin would not have been more effective.

As regards the other children, an intense leukopenia was noticeable during the treatment. In two cases, death occurred after three to five weeks' treatment. In the other case, cortisone effected a complete remission (case No. 21).

*Acute leukemia of children resistant to other therapies.* We here include cases of acute leukemia previously treated with cortisone or antifolics and resistant to these therapies. Treatment with 6MP is often started when the child is in a precarious condition and has reached an advanced stage of the disorder.

The total results are: number of cases, 13; nonvalid cases, 2; valid cases, 11; complete remissions, 3; satisfactory remissions, 1; incomplete remissions, 0; improvements, 1; failures, 6.

*Nonvalid observations.* In two cases, death occurred on the 6th and on the

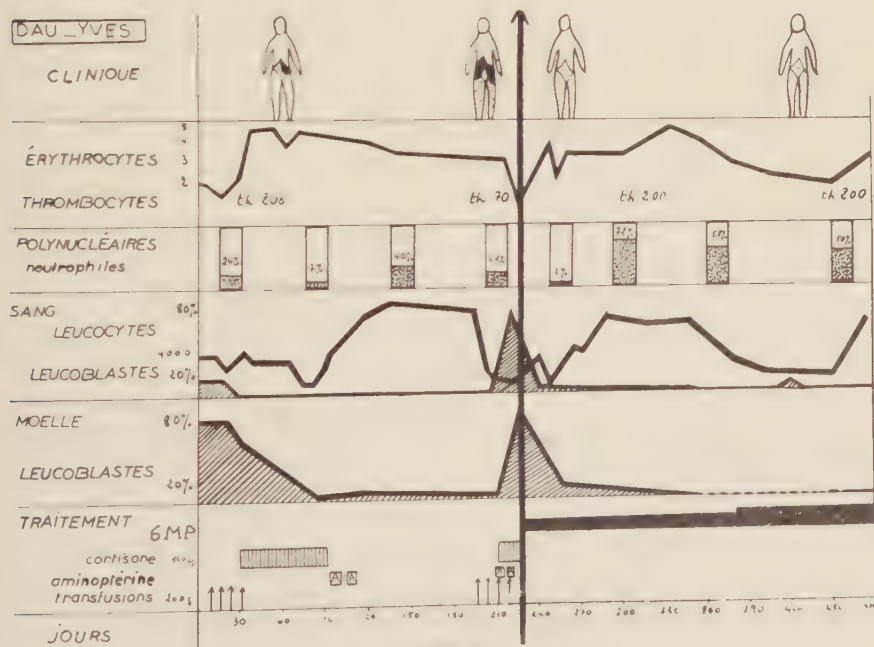


FIGURE 4

TABLE 5

GLOBAL RESULTS CONCERNING THERAPY WITH 6-MP OF ACUTE LEUKEMIAS IN ADULTS

	Cases	Complete remission	Satisfactory remission	Incomplete remission	Clinical improvement	Failure
Untreated previously . . . .	5			1	2	2
Resisted other therapies . . .	3		1			2
Total . . . . .	8	0	1	1	2	4

13th day of treatment respectively. In the first case (No. 41) the number of leukocytes rose from 200,000 to 900,000 (cubic mm.) in six days.

*Complete remissions.* We observed three complete remissions, which respectively occurred 27, 35, and 36 days after beginning treatment with 6-MP. In one of these cases cortisone had first brought on a complete remission; then, after a relapse, it no longer had any effect.

In the second case, a combined treatment of cortisone and Aminopterin had obtained a complete remission in a first attack of leukemia, but in a relapse it obtained only a very incomplete improvement.

In the third case, cortisone at first effected a remission. A relapse did not respond to cortisone or to Aminopterin. The 6-MP treatment was started when the child was in a very serious state. The leukopenia pre-existent to the treatment increased and reached 200 leukocytes/cu. mm. The marrow remission became complete before the number of leukocytes was normal again, which happened only eight weeks after beginning the treatment.

The remissions obtained in this way in these three cases lasted respectively 45 days, 2 months, and 8 months.

Relapses occurred in the three cases. In one case, the administration of 6-MP had been discontinued; in the other two cases treatment with 6-MP was continued. Two of these children have died. One is still alive.

*A satisfactory remission.* We mention here the rather particular case (No. 33) of a child whose clinical condition was very poor, in whom, during a typical "leukemic" relapse which failed to respond to cortisone and to Aminopterin, we obtained, by using 6-MP, a complete marrow remission contrasting with persistent anemia, leucopenia, and slight blood leukoblastosis. The relapse occurred three weeks later, under treatment.

*Improvement.* After two month's treatment with 6-MP, improvement occurred in an infant whose leukemia had become aggravated under treatment with cortisone and transfusion.

*Six failures.* In one case (No. 35) the 6-MP treatment was continued for nine weeks. With the other five patients, treatment was interrupted by death, which occurred within three to eight weeks. All were cases of relapses totally unresponsive to other medications. Death occurred four times in leukopenia and twice in patients experiencing a "leukemic" state.

Special mention must be made of case No. 40. The relapse, at first, was purely meningeal with a high leukoblastosis in the cerebrospinal fluid, the blood remained normal. Treatment with 6-MP did not prevent progressive aggravation, invasion of the blood, and death.

*Acute leukemia of adults.* We treated eight cases of acute leukemia of adults: five cases of acute leukemia not previously treated and three cases of acute leukemia resistant to other therapies.

*Acute leukemia not previously treated.* Results in this case are: number of cases, 5; improvements, 3; and failures, 2.

In three patients, no remission of the marrow was observed. We obtained an improvement in the clinical condition and in the blood without effecting any change in the marrow. The amount of blood polymorphonuclear neutrophils reached 41 per cent in the first case, 44 per cent in the second, and 28 per cent in the third.

One of these patients, a medical doctor, experienced sufficient improvement to be able to live an almost normal professional life for four months, though his marrow remained almost completely leukoblastic.

In another case (No. 43), 6-MP had obtained only a very transitory remission of blood and of clinical condition. Later, Aminopterin produced complete remission of the marrow.

*Acute leukemia resisting other therapies.* Totals in this category include: number of cases, 3; satisfactory remission, 1; failures, 2.

Leukemia in a 20-year-old girl (case No. 47), unresponsive to cortisone, was definitely improved with 6-MP. Marrow leucoblastosis decreased from 79 per cent to 18 per cent. This remission lasted only one month.

In two other cases, progress of the disorder was not modified by 6-MP.

*Myeloblastic transformation of chronic myeloid leukemia.* Results obtained in treatment of the myeloblastic transformation of chronic myeloid leukemia appear remarkable: number of cases, 8; complete remission, 3; satisfactory remission, 1; improvements, 3; failure, 1.

Complete remissions were observed in case No. 51 after 30 days of treatment with 6-MP and after 20 days of treatment with 6-MP in cases Nos. 52 and 53. In the three cases, the marrow became quite normal again, the proportion of myeloblasts being respectively 3 per cent, 6 per cent, and 7 per cent. In one case, the clinical amelioration was not altogether satisfactory. The patient remained rather tired in spite of the remission of the marrow.

The remissions lasted for two months in the two cases in which the treatment was continued properly. The remission did not exceed a few days in the case in which the patient spontaneously stopped the treatment in spite of our advice.

Very similar to these *complete remissions* was the *satisfactory remission* observed in the case of another patient. The proportion of myeloblasts in the

TABLE 6  
MYELOID CHRONIC LEUKEMIAS ON ACUTE MYELOBLASTIC TRANSFORMATION TREATMENT WITH 6-MP

Cases	Complete remission	Satisfactory remission	Incomplete remission	Clinical improvement	Failure
8	3	1	0	3	1
	4				



marrow fell from 67 per cent to 14 per cent after 20 days' treatment. This remission lasted for two months.

*Improvements.* Improvements that were noticed in three other cases are not to be neglected. In a 28-year-old woman, blood myeloblastosis fell from 43 per cent to 5 per cent, while the marrow myeloblastosis remained unchanged.

In a 20-year-old girl (case No. 56), a remarkable amelioration in blood was noticed. The marrow could not be studied. A hemorrhagic syndrome of ambiguous interpretation was the eventual cause of death.

In an 11-year-old child, 6-MP produced a remarkable improvement in clinical condition and blood (the proportion of myeloblasts in blood falling from 75 per cent to 1 per cent). Intercurrent measles suddenly caused the death of the patient.

On the whole, *only one failure* was recorded in the treatment of one of the severest blood disorders.

*Chronic myeloid leukemia.* Totals in this category are: number of cases, 3; complete remissions, 2; failure, 1.

We treated only three cases of chronic myeloid leukemia in the chronic period.

The cases of complete remission were one of leukemia not previously treated, and one of leukemia which was resistant to X rays and urethane. Remission in both cases was obtained in three weeks. These remissions lasted 70 and 20 days respectively. The relapse in the first case (leukemia not previously



FIGURE 5

TABLE 7  
MYELOID CHRONIC LEUKEMIAS TREATMENT WITH 6-MP

Cases	Complete remission	Failure
3	2	1

treated) resisted treatment with 6-MP and responded to X rays. The second relapse showed improvement again under treatment with 6-MP, which again produced a complete remission lasting one month.

The case of failure observed was in the treatment of chronic myeloid leukemia resisting X rays.

### Discussion

Our observations, together with the first reports of Burchenal, confirm the antileukemic action of 6-MP. This action is, to a certain extent, analogous to that of other antileukemic substances, but differs in other respects.

*Common characteristics.* (1) As in the case of exchange transfusion, antifolics, cortisone, and ACTH, 6-MP can produce complete remission in acute leukemia. Not only are clinical and blood disorders corrected, but the marrow, achieving a state of perfect balance, becomes indistinguishable from normal marrow. Even a retrospective diagnosis of leukemia then becomes impossible. This total though transitory reversibility of acute leukemia in man, under the influence of some therapies, has not been observed, using the same therapies, in cases of leukemia in animals. Only a few privileged therapies produce such effects in man.

(2) As in the case of other treatments, the very remarkable remissions obtained with 6-MP are inconstant. Out of 34 cases of acute leukemias (adults and children) not previously treated, we had 12 remissions, or 35 per cent. The proportion is almost the same as that obtained with other therapies (the same criterions of remission being used).

(3) Therapeutic remissions are far more frequent in children than in adults. This conclusion, already established as regards antifolics and cortisone, is true of 6-MP also:

	Valid cases	Complete remissions	Satisfactory remissions	Total
Children.....	34	11	5	16 (48%)
Adults.....	8	0	1	1 (13%)

(4) The number of remissions does not seem to depend on the clinical form of acute leukemia. It depends, to some extent, on the type of cells. The remarkable influence of 6-MP on a myeloblastic attack of chronic myeloid leukemia will be dealt with later, but this effect has no corollary as regards acute myeloblastic leukemia. In the case of 6-MP, we noticed a greater responsiveness on the part of leukemia with blasts and without granulations, and a more frequent resistance on the part of leukemia with granulous blasts.

TABLE 8

DURATION OF TREATMENT WITH 6-MP IN ACUTE LEUKEMIAS BEFORE REMISSION

Shortest.....	28 days
Average.....	30 to 40 days
Longest.....	90 days

TABLE 9

DURATION OF REMISSION IN ACUTE LEUKEMIAS TREATED WITH 6-MP

Shortest.....	21 days
Average.....	2 to 3 months
Longest.....	8 months

(5) The length of remissions is indicated on TABLE 9. As far as a very short period of observation allows us to judge, the duration of these remissions can be compared favorably with those observed when using cortisone and ACTH, but then duration is somewhat shorter than that of remission obtained sometimes with antifolics.

(6) The complete disappearance of any initial sensibility and the development of a secondary resistance were noticed in the use of 6-MP as distinguished from the use of other treatments. On several occasions we had examples of a relapse while treatment was being continued, and sometimes an increase in dosage brought no improvement.

The time for observation which we have had since 1953 has been too short to allow a more definitive study of this resistance. We do not as yet know how often successive intermittent treatments with 6-MP should be applied in order to obtain remissions in the same patients.

*Particular characteristics of 6-MP.* (1) Remissions induced by 6-MP are slow, as TABLE 8 shows. We observed no remission before the 28th day, and sometimes had to wait until the 90th day before observing any change. The average time of therapeutic action was 30 to 40 days. This invariably long delay may be contrasted with the rapidity of effect (10 to 15 days) of antifolics, and with the variable periods of time required by cortisone, ranging from extreme rapidity (sometimes four or five days) to a rather lengthy period (30 to 35 days).

These long-delayed effects of 6-MP can have serious consequences in some cases of leukemia. Previous transfusions, according to the method we advocate, can prevent such consequences to some extent. In appreciating therapeutic results, this slow action must be taken into account. Improvement and remission can occur when these effects are no longer expected.

(2) The disadvantage of this delay and the lengthy duration of treatment required are made up for by the very slight toxicity of the product. We treated 61 patients, observing no serious accident which could be imputed to 6-MP with certitude. We observed a hemorrhagic condition with thrombopenia of ambiguous etiology. Neutropenia, ambiguous also, usually had no clinical expression. The small number of disorders occasioned by the drug may be attributed to the previous systematic application of transfusions used

TABLE 10  
CLINICAL MANAGEMENT OF 6-MP AS COMPARED WITH OTHER TREATMENTS USED IN ACUTE LEUKEMIAS

Practical difficulties incidents and accidents in the follow up	Cytotoxic drugs		Noncytotoxic procedure (moderators)		
	Antifolic	6-MP	Exchange transfusion	Cortisone ACTH usual dosage	Cortisone over dosage
Management	Easy	Easy	Rather impractical; requires numerous blood donors, etc.	Easy	Easy
Accidents	Comparatively frequent	Very rare, almost none	Few	Rare	Surprisingly rare
Patient's condition while under treatment	Fatigue and discomfort increased while under treatment	Unchanged	Poor in the beginning, afterward good	Swelling of the face	

in our treatment. More generally, 6-MP seems to have an important elective effect on leukocytes and a subsidiary effect on red cells and thrombocytes. This difference could be expected from the experimental data and was confirmed by the clinical facts.

As concerns this action, 6-MP proved superior to the other antileukemic treatments. Each method was tested under heavy disadvantages: in vasomotor disorder treated with exchange transfusions; in occurrences—at times very dangerous occurrences—of attack by Aminopterin; in edema of the skin and viscera; and when a deep transformation in the aspect of the patient had occurred resulting from the use of cortisone. Treatment with 6-MP takes, of course, a long time, but it is well tolerated; and it enabled us to induce remission in a child whose appearance remained normal.

(3) The mode of action of 6-MP differs from that of other antileukemic substances. Two clinical facts verify this conclusion:

(1) An acute leukemia which resists cortisone or antifolics may respond to 6-MP, and may do so reciprocally. For instance, in case No. 10, a child, cortisone and Aminopterin had no effect; 6-MP produced a complete remission, a most remarkable effect after a 9-months' development of leukemia.

Sometimes the first drugs produced only a partial remission which 6-MP made complete. Inversely, in the case of a young man (case No. 43), 6-MP and cortisone were ineffective, and Aminopterin produced a complete remission.

The study of associated medicaments is most interesting, as we showed in 1951 regarding cortisone and antifolics. As regards 6-MP, we have so far methodically studied only associated treatments immediately successive, or successive at long intervals. Our experience of double associated treatments (6-MP + cortisone or 6-MP + Aminopterin) or triple (6-MP + cortisone + Aminopterin) is neither long enough nor extensive enough to be taken into account. Let us note only that a triple treatment has so far been well tolerated.

(2) Above all, and this is its most original effect, 6-MP can modify the development of a *very severe myeloblastic attack in chronic myeloid leukemia*. There



TABLE 11

Action upon Leukemias	Cytotoxic Drug		Noncytotoxic Procedure (moderators?)		
	Antifolics	6-MP	Exchange transfusion	Cortisone-ACTH (normal dosage)	Cortisone (over dosage)
Acute leukemia of the child Frequency of complete remission Duration of treatment before re-mission Average duration of the remission Eventual sensitivity to the same treatment	30% 10 to 15 days 2 to 6 months 2 or 3 remissions are possible Quite frequent Very rare None	35% 30 to 40 days 2 to 4 months ? Quite frequent Very rare Frequent	22% 10 days 1 to 2 months Rare Quite frequent Rare None	30 to 40% 4 to 35 days 2 to 9 months 2 or 3 remissions are possible Quite frequent Rare None	Unknown Unknown Possible (frequency unknown) Possible (frequency unknown)
Acute leukemia of the adult: remission to other treatment					
Myeloid chronic leukemia in myelo-blastic transformation: remission					

TABLE 12  
THERAPY OF LEUKEMIAS WITH 6-MP  
*Practical Indications and Contraindications*

	Absolute indications	Reasonable indications	Contra-indications
Myeloid chronic leukemias			
Early stage		+	
On acute myeloblastic transformation	+++		
Acute leukemias			
"Resistant" to other therapies	+++		
not previously treated			
1. dramatically acute development			
2. acute or subacute development		++	+
3. ambiguous development		+	
		(Possibly 6-MP associated with cortisone)	

is no need to dwell at length on the seriousness of this accident, or on the nearly total inefficacy of all known therapies. As we have demonstrated recently, only very high dosages of cortisone can sometimes induce a remission. But even the effect of such doses, important from the point of view of physiopathology, is not certain.

The favorable action of 6-MP was observed in seven of the eight cases treated. In three cases, complete remissions occurred.

The delay of action, about three weeks, is not so long here as in the case of leukemia. Paradoxically, clinical improvement sometimes is not so rapid and complete as improvement of the marrow. The modalities of this treatment, and of its time of application should, no doubt, be more precisely defined. But the quality and importance of the results thus far obtained have already been stressed.

*6-Mercaptopurine in Blood Therapy.* The place that 6-MP will have in blood therapy cannot yet be exactly defined. Its place will certainly be important.

(1) 6-MP is the only therapy, or almost the only therapy, for a myeloblastic attack in chronic myeloid leukemia.

(2) In the course of a chronic myeloid leukemia not transformed, 6-MP can be useful in replacing other therapies.

(3) Together with antifolics and cortisone, 6-MP has its place among treatments of acute leukemia.

The use of 6-MP is definitely indicated in cases of acute leukemia resisting other therapies. As concerns acute leukemia not previously treated, its use depends on the clinical condition and on the imminence of serious danger.

In the case of acute leukemia of short development, or "galloping" leukemia, rapid methods (cortisone, antifolics) should be preferred to 6-MP, the action of which is too slow.

In the common type of leukemia, administration of 6-MP is probably the best initial treatment. It is easier to slow down the development of a recent acute leukemia than that of the often exceptionally acute development of a

relapse, and it is better to keep quickly acting medications for use in case of a relapse. The innocuousness of 6-MP and the small number of accidents resulting from its use speak in its favor when a choice has to be made among different treatments of acute leukemia.

Indications for the use of 6-MP can only be sketched. The spontaneous development of acute leukemia often is irregular. It is not always easy, at first, to judge whether it will be possible to repress such a disorder with palliative treatments until the action of 6-MP can be tested. In such ambiguous cases, the association of 6-MP with some other antileukoblastic such as cortisone seems to be best indicated. The method and order of the different associations are the subject of our most recent researches and cannot yet be given with precision.

### Summary

*Description of the treatment of 61 cases of leukemia with 6-MP.* These cases included 42 acute leukemias of children; 8 acute leukemias of adults; 3 chronic myeloid leukemias; and 8 chronic myeloid leukemias in myeloblastic transformation.

*Treatment.* (1) Blood transfusions, applied until the count of erythrocytes amounted to 4,000,000/cu. mm. (2) 6-MP at a dose of 2.5 mgm./kg. at least nine weeks and, at the utmost, seven months. Therapeutic accidents are exceptional (possibly on account of the previous blood transfusions). A latent leukopenia not infrequently occurs, yet with no clinical consequences generally.

*Results.* (1) A complete remission (bone marrow absolutely normal) in 35 per cent of cases of acute leukemia of children previously not treated; (2) an occasional remission in cases of acute leukemia of children grown resistant to antifolics and cortisone; (3) unsatisfactory results in acute leukemia of adults; (4) satisfactory remissions in certain cases of chronic myeloid leukemia; and (5) a remarkable remission in a very serious acute myeloblastic transformation of acute chronic myeloid leukemia.

The slow action of 6-MP is insisted upon, and its indication is calculated, in relation to this slow action, to the innocuity of the drug and to the results obtained.

The use of 6-MP is definitely indicated (1) in cases of myeloblastic attack of chronic myeloid leukemia; and (2) in cases of acute leukemia resistant to other forms of therapy.

As regards acute leukemia of children not yet treated, the following schema can now be proposed; (1) usual form, 6-MP; (2) very acute form, the association of antifolics with cortisone; and (3) ambiguous forms whose evolution it is difficult to foresee, the association of 6-MP with cortisone.

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## THE TREATMENT OF BLASTIC LEUKEMIAS WITH 6-MERCAPTOPURINE

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Our experience in the therapeutic use of 6-mercaptopurine (6-MP) refers only to the blastic leukemias. We use this latter term because we think that to continue referring to processes that can last two or more years as acute leukemias is inappropriate. Nor do we define the classification of myeloblastic and lymphoblastic leukemias, because nearly 40 years of experience have convinced us that the undifferentiated cell of the greater part of the so-called acute leukemias is a particular neoplastic cell that can be identified only exceptionally with any normal undifferentiated cell (myeloblast or lymphoblast) and could be called "paraleukoblast" or just simply "blast." Thus would be avoided an error that many hematologists fall into and one into which we also have sometimes fallen, namely, diagnosing as lymphoblastic leukemia a case which later has changed, maturing in a myeloid sense, having acquired under treatment a subacute character and having partially filled the leukemic hiatus. Because of such potentialities we think it questionable to affirm that such and such a drug is more or less effective in treating lymphoblastic or myeloblastic leukemias.

Another comment we deem necessary is to indicate the general method followed in treating blastic leukemias, a process which is carried out in three stages (FIGURE 1):

(1) A stage of attack or provocation of a reversible aplasia is produced with antimetabolites (actually with 6-MP). For this purpose, the drug is administered daily until total disappearance of the blasts is achieved, a stage accompanied by a marked leukopenia that, in some cases, may reach 800 to 1000 leukocytes. At this level, administration of the antimetabolite is discontinued.

(2) A stage of recuperation or stimulation of the orthoplastic leukopoiesis follows. In the more fortunate cases this stage spontaneously succeeds the first, but when it is delayed we hasten it with cortisone or ACTH.

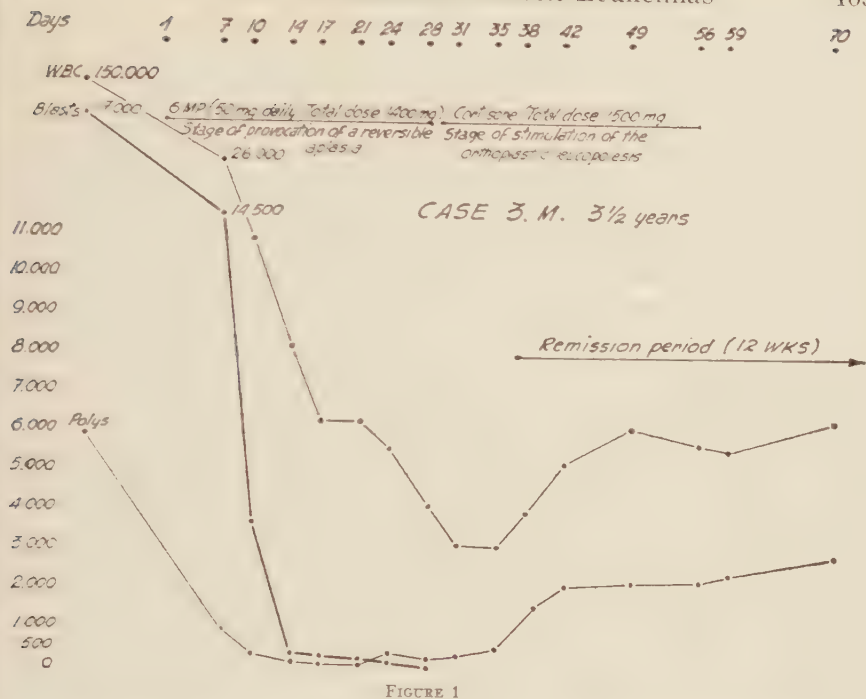
(3) A remission follows, during which we have tried different methods: careful observation without drugs, the administration of alphetocopherol or of antimetabolites in small doses or, in normal doses, two or three days a week. The procedure to follow during remissions is of such interest that later we shall return to it.

*Cases treated with 6-MP.* Not including three new cases whose treatment we have just initiated, our experience with 6-MP refers to 16 cases: six children previously untreated; five children in relapse previously treated with Aminopterin, A-Methopterin, and/or cortisone; and five adults.

In TABLES 1, 2, and 3 we have summarized details of each case.

We wish to enlarge upon the following points: doses, toxicity, results ob-

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tained, comparison with results obtained with folic acid antagonists, resistance and sensitivity to the antimetabolites, combined and simultaneous treatment with folic acid antagonists, with 6-MP and hormones (cortisone or ACTH), and the procedure followed during periods of remission.

**Dosage.** Generally, we have administered approximately 2.5 mgm./kg. of body weight when starting treatment. The response to the drug, measured by the decrease of the leukocyte count in general and of the blasts in particular, has been our criterion, and if the decrease has been delayed we have encountered no accidents in administering a greater dose, or 4 or more mgm./kg.

**Toxicity.** In none of the 16 cases have we observed important toxic phenomena directly attributable to 6-MP, at least during treatments that have not been too prolonged. A decrease in the leukocyte count is the prerequisite condition for a remission, and it cannot be considered as a disturbing toxic phenomenon. In one of our cases (No. 8), a patient who had already suffered several relapses, we found, in the few days that preceded death, a leukopenia so marked that it nearly reached a total disappearance of leukocytes from the peripheral blood, but this hematic change, which we have seen in other untreated leukemic patients as a terminal phenomenon, perhaps cannot be imputed in this case to 6-MP, as the dose administered at the time was very small. The profuse and stubborn diarrhea of another of our cases (No. 10) was not attributable to the drug. Vomiting and diarrhea preceded administration of 6-MP and persisted for a long time after discontinuation of the drug; the total

TABLE 1

Case no.	Age (yrs.) sex	Status before 6-MP therapy				6-MP therapy		Peripheral blood on suspending treatment with 6-MP				Treatment with cortisone immediately following	Time past the beginning of treatment till start of remission (wks.)	Duration of the first remission (wks.)	Response to 6-MP in the relapse	Duration of the survival from the beginning of the disease till this moment (wks.)	
		Clinical particularities	Peripheral blood				Duration days	Total dose (mg.)	Peripheral blood								
			RBC (mill.)	WBC (th.)	Blasts %	Polys. %			RBC (mill.)	WBC (th.)	Blasts %						Polys. %
1	7 F	Micropoliodenopathy, spleen palpable Micropoliodenopathy, pain in the joints Micropoliodenopathy, splenomegaly	2,0	4,2	15	2	38	1.450	4,8	1,6	0	33	1.500 mg. in 20 days	7	22	A satisfactory second remission is produced	40, 5
2	3½ M		1,8	1,6	1	2	26	575	3,8	4,6	0	20		4	20	The relapse is now developing	30
3	3½ M		2,2	150,0	56	4	28	1.400	3,6	4,2	0	2	1.500 mg. in 28 days	5	12	The second remission is lengthened now 10 weeks. Still continuing (1150 mg. 6-MP in 23 days).	35
4	5 F		3,7	18,2	83	4	16	800	4,8	0,4	0	0	2.000 mg. in 30 days	4	3½	The second remission is lengthened now 5½ weeks. Still continuing (2 series of 800 mg. 6-MP with an interval)	27
5	5 M	Micropoliodenopathy, pain in the joints	3,6	64,2	80	2	21	1.300	4,4	2,0	1	1	2.000 mg. in 30 days	4½	5½	The second remission is lengthened now 6 weeks. Still continuing (1975 mg. 6-MP in 2 series with an interval).	22
6	6 M	Micropoliodenopathy, huge splenomegaly	2,8	120,0	86	1	36	2.700	3,8	78,0	70	2				The parents decide not to continue the treatment	

amount administered to the patient was very small. We wish to point out also that in three cases (Nos. 4, 10, and 13) we have observed the appearance of a high relative neutrophilia (total number of leukocytes inferior to normal or within normal limits) accompanied by the disappearance of blasts and by marked changes in the neutrophils (abnormal nuclear segmentation, toxic granulations, *etc.*), but we do not venture to attribute these effects specifically to the drug, as they could be consequences of intercurrent infections.

In any case, this marked alteration requires mention, as does the serious leukopenia which accompanied the irreversible aplasia in other case not treated directly by us, in which the administration of 6-MP was prolonged for a lengthy period.

*Results obtained.* This drug, like the folic acid antagonists, is much more effective in children than in adults and, as is natural, its effectiveness is greater in previously untreated cases than in cases already treated, *i.e.* in relapses or cases resistant to other treatments. Among six children previously untreated (Nos. 1 to 6), five remissions were achieved that could be considered total. The remission in case No. 4 lasted three and one half weeks; in No. 5, five and one half weeks; in No. 3, 12 weeks; in No. 2, 20 weeks; and in No. 1, 22 weeks (TABLE 1 and FIGURE 2). In three children treated in a first relapse (Nos. 7, 8, and 10), case No. 7 did not respond to treatment, and case No. 10 showed so many unexpected complications that the hematic remission (disappearance of blasts) could easily have been attributed to intercurrent infection. In case No. 8, however, a second quite satisfactory remission was achieved with 6-MP, although in the following relapses, separated by brief partial remission, the drug was less and less effective. In case No. 9, an adolescent in whom three remissions had already been obtained, 6-MP failed, as had also antifolics and hormones. Case No. 11 (TABLE 2) will be commented on later.

We must add that, in four cases (Nos. 1, 3, 4, and 5), in which we obtained first remissions, we repeated the drug when relapses occurred, and second remissions were obtained. These second remissions continue as this paper is written (FIGURE 2).

In the adults, results have been less satisfactory. In three of them (Nos. 12, 13, and 14), 6-MP did not delay fatality, even though cortisone and antifolics were associated with the drug in two of them. In No. 15, however, whose relapse followed a very unfavorable course, the simple administration of 6-MP effected a clinical and hematic recovery that could be considered a partial remission. Finally, in No. 16, a previously untreated case in whom associated medication was started soon after onset of the disease, the result was so satisfactory that it could have been considered total remission. This remission is still in course after nine weeks (TABLE 3).

*Comparison with results obtained from folic acid antagonists.* The number of cases treated do not seem sufficient to establish a percentage in remissions obtained that could be compared fairly with the percentage obtained by using folic acid antagonists. The proportion of 83 per cent obtained in previously untreated children seems too high, and we think that this percentage may be the artificial result of a run of favorable cases. For the present, we do not venture



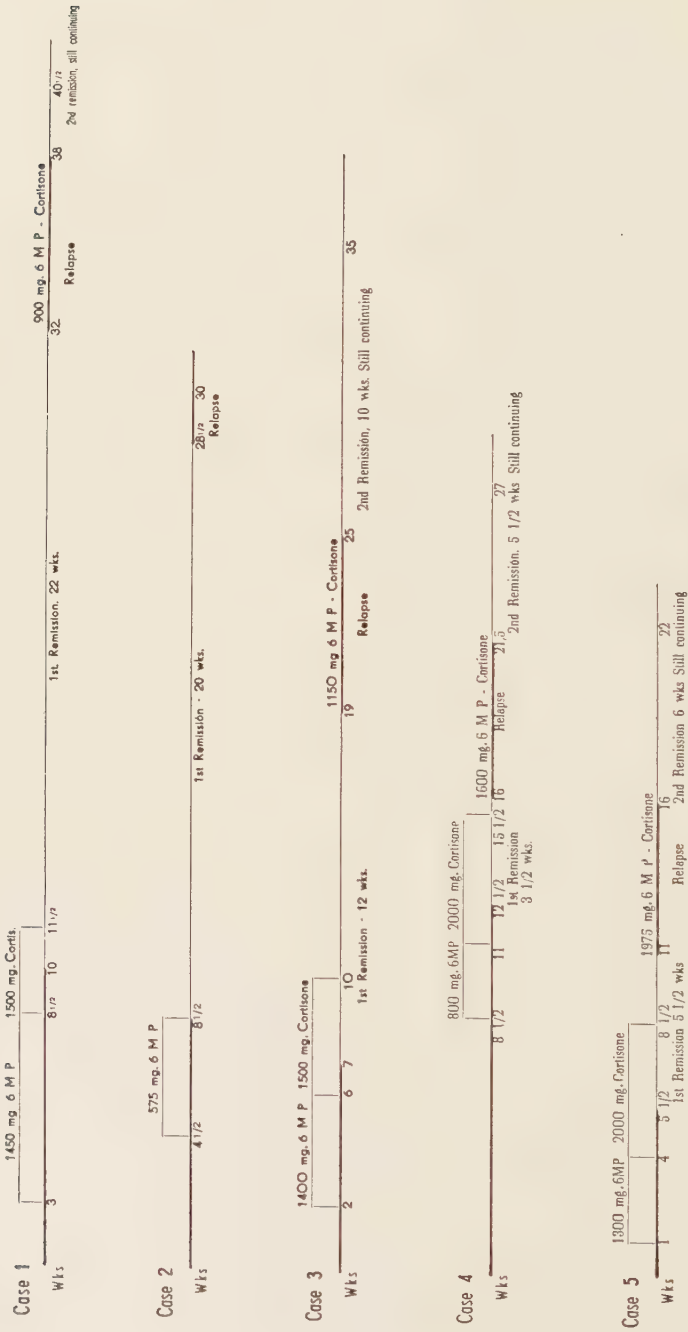


FIGURE 2. Previously untreated children.

TABLE 2  
CHILDREN IN RELAPSE PREVIOUSLY TREATED WITH ANTIFOLIC ACIDS AND/OR CORTISONE

Case no.	Age (yrs.) sex	Previous treatment	Remissions obtained	Time past from begin- ning of the disease till start of 6-MP (wks.)	6-MP therapy			Course of the disease
					Dura- tion days	Total dose (mg.)	Results obtained	
7	3½ F	Aminopterin and corti- sone	1 (9 wks.)	21	25	1.250	None	Death at 6½ months after the beginning of the dis- ease.
8	1½ F	Amethopterin and al- phatocopherol	1 (10 wks.)	17	37	1.850	Remission very satis- factory that it is lengthened about one month.	Relapses treated with 6-MP followed by short remis- sions. Death at 9 months after the beginning of the disease.
9	12 M	Aminopterin, ameth- opterin, cortisone, hydrocortisone	3 (6, 4 and 5 wks.)	44	29	2.900	None, including when A-Methopterin is added.	Death at 12 months after the beginning of the disease
10	3 M	Cortisone	1 (partial with re- lapse during treatment)	13	9	450	Marked leukopenia with disappearance of blasts.	Numerous unexpected com- plications. Enterocolitis, spontaneous pneumotho- rax, abscess in the other lung. ACTH and corti- sone are administered. 5½ months have passed since the beginning of the disease.
11	9 M	Aminopterin (37 days)	None	6½	24	1.250	Remission partial that it is lengthened 4 weeks.	Relapse resistant to 6-MP. Death at 6 months after the beginning of the dis- ease.

TABLE 3  
ADULTS

Case no.	Age (yrs.) sex	Previous treatment	Time past from the beginning of the disease till start of 6-MP	Treatment			Results
				6-MP therapy		Other simultaneous treatments	
				Duration days	Total dose (mg.)		
12	40 M	ACTH intravenous, that did not produce remission.	Many weeks.	20	3,000	—	None. The patient died shortly after suspending treatment.
13	50 M (sub-acute form)	None. The diagnosis had not been established.	Many months	13	1,300	Cortisone and ameth-	The patient died during the treatment.
14	58 F	Cortisone, that did not produce remission.	Many weeks.	12	1,800	ACTH and aminop-	The patient died during the treatment.
15	62 F	ACTH intravenous and aminopterin produce partial remission about 4 weeks.	20 weeks.	20 Still continuing	3,000	Cortisone.	Partial remission.
16	35 F		5 weeks.	44	4,900	Cortisone and aminop- terin.	Remission very satisfactory after 41 days of treatment. In mid remission the patient moves to another city where future treatment will be difficult.

to compare this percentage with the 66 per cent of remissions achieved in 30 children treated with antifolics.

A great advantage in using 6-MP is the absence of toxic phenomena (ulcerous stomatitis, for example, which results so frequently from the use of Aminopterin). This nontoxic quality of 6-MP permits prolongation of treatment for any necessarily extended period. The duration of a remission obtained by using 6-MP does not differ markedly from one obtained by using folic acid antagonists, but we must add that our most favorable case is one treated with antifolics. In this case, a child whose illness began three years ago, when it was 11 months old, we have obtained an apparent cure which has now lasted for six months without treatment.

*Resistance and sensitivity to antimetabolites.* We lack sufficient experience in regard to this important subject.

Case No. 7, whose disease had responded favorably to Aminopterin, showed resistance to 6-MP in a first relapse which took so rapid a course that it left us no time to use the antifolic again. Nor did case No. 9 respond to 6-MP, but here we were dealing with a very unfavorable case that had become resistant to antifolics and hormones following a prolonged course of the disease, during which several remissions and relapses had occurred. As a positive case, we may consider No. 8, a patient who experienced a very satisfactory remission under 6-MP in his first relapse, which occurred during treatment with A-Methopterin. Better still was case No. 11. In this child, treatment with Aminopterin had been discontinued after 37 days because of toxic phenomena, especially as we had no evidence that the drug had substantially improved the clinical and hematic picture. Nevertheless, 6-MP provoked a very satisfactory remission that lasted four weeks.

As to the possibility that patients who had become resistant to 6-MP could be sensitive to an antifolic, we have no experience on which to comment. This point is so important that on it rests the possibility of substantially increasing the survival period of these patients.

*Combined simultaneous treatments with 6-MP, antifolics, and cortisone or ACTH.* Burchenal *et al.* point out the possibility of using the association of drugs in the same manner as in the treatment of tuberculosis. Our opinion is that, in treating children, who respond so well to the antimetabolites, only one drug should be used, in order to avoid the possible appearance of simultaneous resistances to both antimetabolites. As regards adults, in whom, as we know, the proportion of remissions is very small, we think that the association of drugs is very opportune. Our case No. 16 is a good example of results so obtained.

*Procedure during periods of complete remission.* Our experience with antifolics, as well as with 6-MP, inclines us to advise total discontinuation of treatment when the remission is complete. With the idea of incorporating, if possible, regulator substances of the normal hematopoiesis, we administer transfusions not oftener than once a week, and sometimes not that often.

Theoretically, and taking into account the effects we have observed of antibiotics on infections, continued treatment, especially in small doses, should facilitate the production of states of resistance. We therefore think it advisable



to discontinue treatment once the remission is obtained, and to reinstate it at the first sign of clinical or hematological relapse. On the other hand, continuation of treatment during remission places us in the unfavorable position of increasing the dosage considerably when a relapse occurs during full treatment, or of being obliged to change prematurely the antimetabolite in use.

Practice seems to confirm these theoretical considerations. The procedure we suggest has enabled us, by using hormones and antifolics, to obtain an average survival of nearly a year in 20 children who responded favorably, of the 30 children treated. As regards 6-MP, the duration of the remissions obtained with discontinued treatment ( $3\frac{1}{2}$ ,  $5\frac{1}{2}$ , 12, 20, and 22 weeks) does not seem to us inferior in duration to the remissions reported by Burchenal *et al.* in 15 children who responded favorably to treatment (maximum duration in one case, 22 weeks). On the other hand, in a child of five years not treated directly by us, but whose course we followed carefully, the relapse occurred at  $12\frac{1}{2}$  weeks in spite of continued treatment of 50 mgm. of 6-MP, and the patient died in total aplasia, 21 weeks after onset of the disease.

## ONE-YEAR EVALUATION OF 6-MERCAPTOPURINE IN THE TREATMENT OF LEUKEMIA

By Charles A. Doan, K. Wiseman, and Bertha Bouroncle  
*College of Medicine, Ohio State University, Columbus, Ohio*

During the past year, 60 patients having various types of leukemia have received 6-mercaptopurine (6-MP) as complete or partial treatment.

Thirty-three patients afflicted with acute leucolympho-sarcoma, ranging in age from one and one-half to 53 years, have been followed. Eight patients obtained remissions on this drug alone, lasting from two to eight months. Nine patients were carried from one to six months on a combination of 6-MP, Aminopterin, and cortisone. Three patients experiencing remissions induced by Aminopterin obtained further remissions with 6-MP alone lasting two to nine months after resistance developed to Aminopterin and cortisone. One patient obtained a 9-month remission while receiving 6-MP, Aminopterin, and cortisone together. Four patients only, after a fair trial on 6-MP, failed entirely to respond to this compound. Neither Aminopterin nor cortisone helped in these same patients. Eight of the patients succumbed to their diseases after less than 12 days on the drug and therefore may be considered to have had an inadequate trial.

Much less favorable results have been obtained in our clinic with 6-MP as used in acute myeloid and monocytic leukemias. Seventeen patients having a diagnosis of myelogenous leukemia have been treated with 6-MP. One 9-year-old male having a primary acute myeloid leukemia has been in complete remission for 14 months and the remission is still continuing. Another patient had a complete remission for four months. Two others failed to respond. Twelve patients with acute blastic exacerbations of chronic myelogenous leukemia, with an age range of 27 to 66 years, were treated with only two showing a short, temporary improvement. One patient afflicted with chronic myeloid leukemia is currently being treated successfully.

Ten patients ranging between 15 and 78 years of age having acute monocytic leukemia have failed to show any hematologic improvement on this therapy. Toxic symptoms were minimal in this series and will be discussed in detail later.

# SUMMARY OF EXPERIENCE WITH 6-MERCAPTOPURINE

By Sidney Farber

*Children's Cancer Research Foundation, Boston, Mass.*

*Introduction.* These observations on the therapeutic use of 6-mercaptopurine (6-MP) were gathered from studies carried out by the Tumor Therapy Group of the Children's Cancer Research Foundation of Boston under Doctor Sidney Farber. The senior members of this group during the period of study include Ruth Appleton, Marie deRipainsel, Virginia Downing, Robert H. Johnson, James P. King, Ruth M. Phillips, and Rudolf Toch. The statistical analysis was prepared by Doctor Toch.

## 6-Mercaptopurine Summary

Total number of patients started on therapy: 96  
Patients treated for less than 30 days: 16

## Roster of Diagnosis of Evaluated Patients

Acute leukemia.....	60
Chronic leukemia.....	2
Lymphosarcoma w/leukemia.....	6
Lymphosarcoma w/o leukemia.....	1
Neuroblastoma.....	3
Hodgkin's.....	1
Wilms' tumor.....	2
Hand-Schüller-Christian.....	1
Collagen disease.....	1
Miscellaneous tumors.....	3

Age groups: under 15 yrs.: 71  
15 yrs. and over: 9

Age range: 10 months to 62 years

Weight range: 8 to 77 kg.

Daily dose range: 12.5 to 200 mgm.

Largest total amount given: 9880 mgm.

Longest period of therapy: 162 days

Range of mgm./kg./day doses: 0.93-5.0

*Frequency of Average Daily Doses (mgm./kg.).*

Used: <1.0 : 1  
1.0-1.4: 5  
1.5-1.9: 12  
2.0-2.4: 19  
2.5-2.9: 27  
3.0-3.9: 12  
>4.0 : 4

## Acute Leukemia.

*Complete remissions:* 9/60 (15 per cent)

Without other therapy 1

With cortisone 5

With A-Methopterin 2

With ACTH 1

*Partial remissions:* 19/60 (31 per cent)

Without other therapy 6

With cortisone 9

With A-Methopterin 3

With ACTH 1

*Clinical improvement only:* 4 (6.6 per cent)

*No improvement:* 28 (46.7 per cent)

Without other therapy

12

With cortisone	7
With cortisone and A-Methopterin	8
With ACTH	1

*Interval Between Onset of Therapy and Onset of Remission.*

9 to 70 days (average 30 days).

*Duration of Remissions (Complete and Partial).*

2 weeks	3	10 weeks	1
3 weeks	2	11 weeks	2
4 weeks	2	13 weeks	2
6 weeks	4	14 weeks	1
8 weeks	8	17 weeks	1
9 weeks	2		

Average  $7\frac{1}{2}$  weeks.

Mean 8 weeks

Nine patients had had no previous therapy. Six improved, five of these on combined therapy with A-Methopterin and/or cortisone.

Of 51 patients who had had previous therapy, 10 patients had failed to respond and failed also on 6-MP alone or as addition to other agents.

Nineteen patients who had responded (and later relapsed) failed to respond to 6-MP alone or in combination.

Twenty-two patients who had responded (and later relapsed) responded again to 6-MP alone or in combination.

Five patients who had failed to respond to 6-MP responded to subsequent therapy with other agents, and three patients who had responded to 6-MP and later relapsed, responded to other agents subsequently.

*Chronic leukemia.* Temporary hematological improvement occurred in both patients.

*Lymphosarcoma.* Of six patients having acute leukemia, two showed hematological improvement as well as decrease in the size of lymph nodes. In one patient, skin lesions decreased in size, but the patient converted to acute leukemia while on 6-MP.

*Neuroblastoma; Wilms' tumor; other tumors.* No clear-cut evidence of effect.

*Hand-Schüller-Christian and collagen disease.* No effect.

### Toxicity

*Leukopenia.* Seventy-five per cent of all patients developed marked leukopenia ( $<2000$ wbc) necessitating omission of therapy. Wbc usually rose promptly when 6-MP stopped. No persistent bone marrow depression has been observed.

*G. I. Symptoms.* A few patients developed nausea, vomiting, or diarrhea, which diminished after withdrawal of 6-MP. Stomatitic lesions were noted in only two patients.

*Icterus.* Six patients developed icterus while on 6-MP. Liver function tests inconclusive. Icterus cleared when 6-MP omitted.

### Summary

(1) 6-MP produces hematological improvement in about 50 per cent of patients having acute leukemia.

(2) This improvement represents roughly one third complete remissions and two-thirds hematological improvement.



(3) The average duration of improvement is eight weeks.

(4) Improvement may occur after other therapy has failed to maintain previously induced responses. In our experience, no patient responded to 6-MP who had failed to respond previously to cortisone and/or FAA.

(5) Combined therapy with cortisone and/or FAA was often employed but there is no evidence that the response is more quickly attained or longer maintained than similar responses to single agents. Our data do not show whether such combined therapy will be effective where singly used agents fail, but clinical impression points that way.

(6) As with the FAA, leukopenia during 6-MP therapy of acute leukemia does not always indicate drug toxicity but may represent an early phase of response to therapy. All patients who responded to therapy showed leukopenia.

(7) Gastrointestinal disturbances and possibly icterus are definite signs of toxicity. Stomatitis is rarely encountered.

(8) Some effect has occurred on chronic leukemia and lymphosarcoma—leukemia, but no clear cut effect upon solid tumors has been noted so far.

*Conclusion.* 6-MP is a useful agent within the framework of total care for patients having acute leukemia. Its myelotoxic properties make it dangerous in unskilled hands. Its place in the treatment of solid tumors must yet be determined.

## LEUKEMIA IN CHILDREN: TREATMENT OF 22 CASES WITH 6-MERCAPTOPURINE\*

By Mila Pierce

*Bobs Roberts Hospital for Children, Department of Pediatrics, University of Chicago, Chicago, Ill.*

6-Mercaptopurine was made available for a clinical trial in our chemotherapeutic program for the management of childhood leukemia in January 1953 (see TABLE 1). Twenty-two cases have been treated to date, and 20 of these are now deceased. Nineteen of the 22 cases were acute leukemias, 13 stem-cell, 2 myeloblastic, and 4 monoblastic. Three cases were of the leukoblastic (lymphosarcoma) type. The age range varied from 2 to 13 years, and 15 cases were under 7 years old. Eighteen of the 22 cases were treated, while in relapse after therapy with adrenocortical hormones or folic acid antagonist drugs, and in only four cases was 6-mercaptopurine used as the first antileukemic drug.

The dose employed was 2.5 mgm. per kilogram of body weight per os, once daily, continued until the leucocyte count had fallen 50 per cent or more from the pretreatment level. At this point the drug was withdrawn until the sharpness of the leucopenic trend could be evaluated. When the curve was stabilized, even though at a level of circa 2,000, therapy was resumed. The dose employed proved to cause a leucopenic response, and was tolerated without other signs of toxicity, in 20 of the 22 cases. Stomatitis developed in 2 children. Skin rashes or signs of gastrointestinal intolerance were not encountered.

The drug was continued for 3 weeks or more in 15 cases, and for less than 3 weeks in 7 cases. It was tolerated for more than 3 months in 2 cases; for 6 to 12 weeks in 11 cases; for 3 to 6 weeks in 2 cases; and for only 6 to 15 days in 7 cases. The reasons for withdrawal of the drug were: (1) severe leucopenia; (2) resistance manifested by a persistence of clinical signs of spleno- or hepatomegaly and lymphadenopathy and hematological evidence of an active leukemia state in the blood or marrow; and (3) failure to show clinical or hematological evidence of a response.

The following case histories illustrate some of our experiences with the drug.

*Case 1.* J. McC. Female, age 4 years. Monoblastic leukemia. Weight 17.4 kilograms. Onset December 1951. See FIGURE 1.

This child had responded well to an initial course of ACTH followed by Aminopeterin, and continued in remission with intermittent Aminopterin therapy for the first nine months of the disease. A relapse occurred in September 1952, which was not brought under control with ACTH, A-Methopeterin, or several transfusions. 6-Mercaptopurine therapy was started in January 1953, when her clinical condition was poor. The patient was uncomfortable with bone pain, febrile, and showed a generalized purpura, hepato- and splenomegaly. The hematological status was that of a leukemic pancytopenia. In spite of transfusions the RBC level remained below 3.00 M., and reticulocytes were absent from the blood. Fourteen days after the addition of the 6-mercaptopurine, the clinical condition had improved; the reticulocytes rose to 2.4 per

\* This work was supported in part by Grant No. 34 of the American Cancer Society.

TABLE 1  
LEUKEMIA IN CHILDREN  
TWENTY-TWO CASES TREATED WITH 6-MERCAPTOPYRINE  
(DEPARTMENT OF PEDIATRICS, UNIVERSITY OF CHICAGO)

Stem cell type.....	13 cases
Myeloblastic.....	2
Monoblastic.....	4
Leucosarcoma.....	3
Age range less than 3 years.....	4
3 to 6 years.....	11½
7 to 10 years.....	4
10 to 13 years.....	3

cent and continued to rise to 5.8 per cent. The return of granulocytes to the blood, and the rise in the platelet level indicated that a marrow remission had occurred. The drug was continued daily and on April 4, a bone marrow confirmed the clinical finding that the remission was sustained.

In June, a relapse occurred while the drug was still being administered, and further remission could not be obtained with either ACTH or A-Methopterin. The child expired in July, 19 months after the onset of her illness. It was felt that the use of the 6-mercaptopurine extended the life of this patient several months.

Case 2. E. G. Age 8 years. Weight 22 kilograms. Stem-cell leukemia. Onset July 1952 (See FIGURE 2).

This eight-year-old boy was treated initially with ACTH in July 1952, within

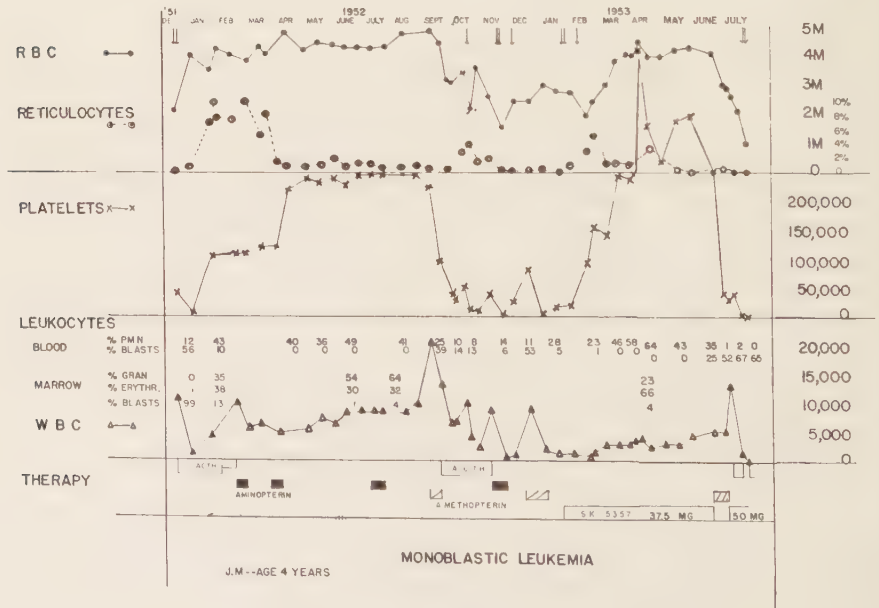


FIGURE 1

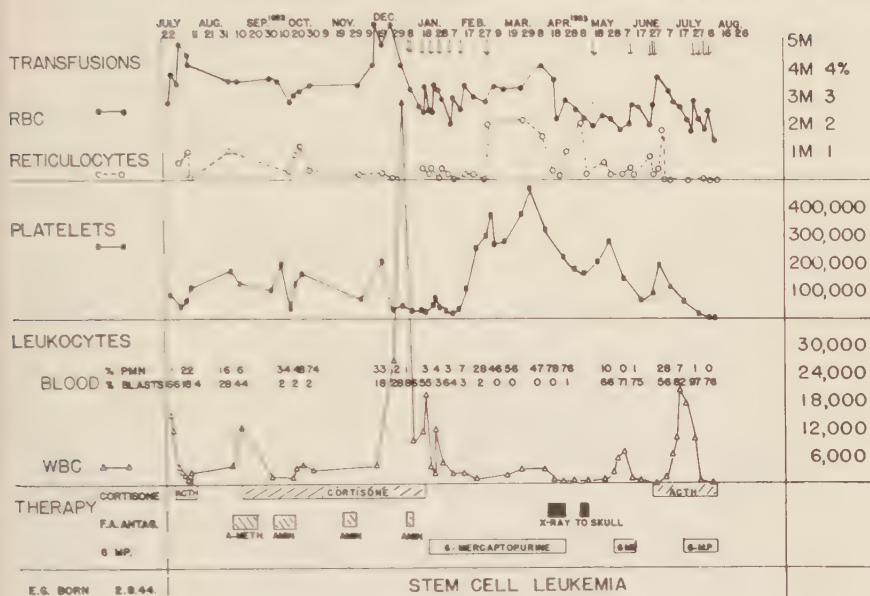
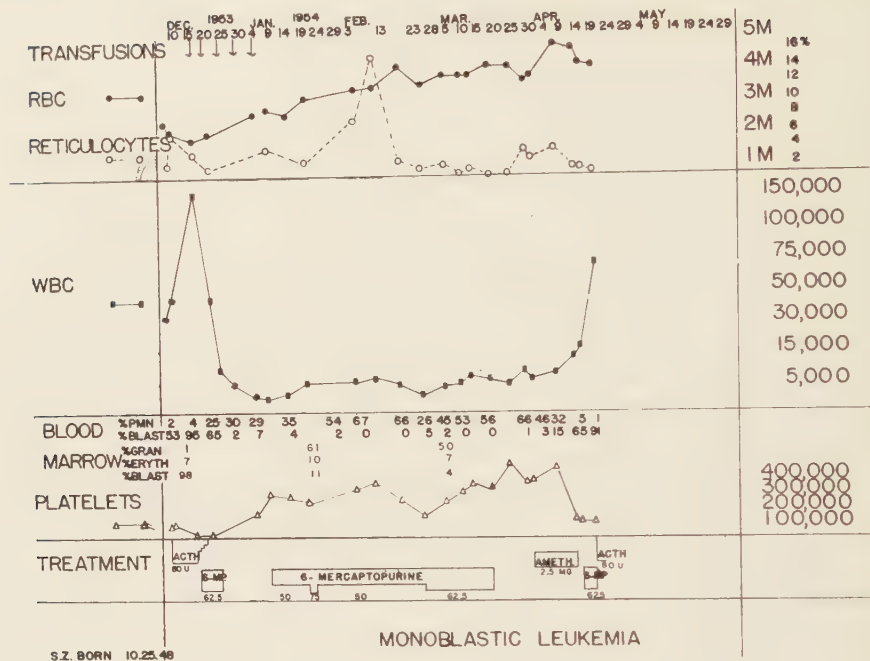


FIGURE 2

a week after his disease was recognized, and a remission resulted which lasted only four weeks. A-Methopterin and later Aminopterin therapy kept the disease under partial control until October 15th, and although a complete remission was not obtained, he was ambulatory and comfortable at home. Cortisone therapy was instituted October 15 and continued to January 15, without a complete remission. About December 20, the RBC dropped, and the patient gradually developed progressive signs of purpura, lymphadenopathy, enlargement of the liver and spleen, bone tenderness, and X-ray evidence of leukemic periostitis. The WBC rose to 83,000 on December 31. After transfusions and another course of Aminopterin, it fell to about 10,000 on January 9.

On January 17, 6-mercaptopurine 62.5 mgm. (2.5 mgm. per kg.) was started. The patient was then in poor clinical condition, uncomfortable with bone pain, and showed a severe bleeding tendency, thrombocytopenia, anemia, hepatomegaly, and splenomegaly. The WBC was 9900 with 55 per cent blasts, reticulocytes were absent from the blood and the platelet count was 23,000. On February 9, the WBC had fallen to 1200, but the drug was continued without interruption until March 25. A clinical remission was obtained. Improvement was evidenced by freedom from pain and a general sense of well being. The liver and splenic enlargement receded, although the spleen remained palpable at the costal margin; and the child again became ambulatory. The platelet count returned to normal. Reticulocytosis and the reappearance of granulocytes indicated the marrow response, although a marrow examination was not done at this time.





While the clinical remission was still in evidence, except for the palpable spleen, and while no blasts were detected in the blood, the patient developed a severe headache and meningeal signs. A lumbar tap revealed a cell count of 1,250, all of which were blast forms. A severe pilledema appeared, but no infiltrates or hemorrhages were to be seen in the retinae. The patient was treated with X radiation to the base of the skull, with subsequent disappearance of the signs of increased intracranial pressure. Following the radiation therapy, a severe leukopenia 660 developed, and it was not considered wise to continue the 6-mercaptopurine alone. A course of ACTH was given together with the drug, and a transient leukocytosis developed, but the terminal stage of the disease ensued with a pancytopenia.

*Case 3.* S. Z. Age 5½ years. Monoblastic leukemia. Onset December 1953. (See FIGURE 3).

This child, having a monoblastic type of leukemia of recent onset, was treated initially with a course of ACTH, 80 units daily for 10 days without clinical improvement or any favorable change in the blood findings. Hormone therapy was discontinued, and 6-mercaptopurine, 3 mgm. per kg., when the WBC was 148,000 with 95 per cent blasts, was started on December 17, 1953. Unfortunately, transfusions were also necessary at this time because of the degree of anemia and purpura. On December 19, the dose was reduced to 2.5 mgm. per kg. On December 24, the WBC had fallen to 8900, and the drug was discontinued. Four days later the leucocyte level had stabilized at 2950, the

percentage of granulocytes had risen to 34 per cent, and the blasts had dropped to 4 per cent.

The drug was started again at 2.0 mgm. per kg., and continued at this dose throughout the patient's remission. By January 20, the marrow remission was good, with a differential count of: granulocyte, 61 per cent; blasts, 11 per cent; and erythroid elements, 10 per cent. The clinical and hemotological remission was sustained throughout February. The dose was increased to 62.5 mgm. on February 25, when 5 per cent blast appeared in the blood. Although the marrow still showed a good marrow remission, the spleen began to enlarge against this time.

On March 4th, azaserine, 50 mgm., was added after two weeks of the combined therapy. The patient developed a moderately severe stomatitis, and all therapy was stopped. Terramycin had been used in doses of 250 mgm. b.i.d. throughout the remission, and this treatment was also stopped. (Culture of the mouth failed to reveal a monilial stomatitis, and the clinical appearance did not suggest a moniliasis.) After recovery from stomatitis it was thought best to change from 6-mercaptopurine to amethopterin. However, after six days of treatment with this drug, the percentage of blasts continued to rise, and before its effect could be evaluated, the child developed measles, and his treatment shifted back to 6-mercaptopurine through this illness. The course of measles was mild, but the leukemia has been out of control for the past three weeks, and a course of ACTH is being given at present.

*Case 4. E. C. Age 4 years. Weight 22 kilograms. Myeloblastic leukemia. (See FIGURE 4).*

This four-year-old boy with an acute myeloblastic leukemia of recent onset was treated initially with transfusions, cortisone followed by ACTH, and 6-mercaptopurine, 50 mgm. daily. A clinical remission followed. He came under our care about the time that a relapse was evidenced by a rise in the WBC to 251,000, and a return of blasts to the blood. A bone marrow examination on November 13 revealed a cellular marrow with a predominance of stem cells, myeloblasts, and promyelocytes.

After the initial course of ACTH, 6-mercaptopurine, 50 mgm., and cortisone, 50 mgm., had been given daily and, on November 20, the cortisone was discontinued to determine whether a better effect could be obtained on the drug alone. The leukocyte count fell to 10,000, and the platelet level to 100,000, but the percentage of blasts remained at about 30 per cent. On December 15, acaserine, 50 mgm., was added, but no fall in the percentage of blasts occurred. On January 3 the WBC rose to 56,000, blasts to 78 per cent, and a course of ACTH was started. No benefit was noted in the hematological picture. The leukocyte count continued to rise and the platelets continued to fall. The drugs were stopped on January 15, and the ACTH therapy alone continued.

On January 23, a mild hypertension of 130/90 mm. developed; ACTH was tapered off and stopped on January 27. On the following day, the child had a sudden intracranial hemorrhage and died.

*Case 5. R. M. Age 13 years, weight 72 kilograms. Stem-cell Leukemia. Onset, December 1952. See FIGURE 5.*

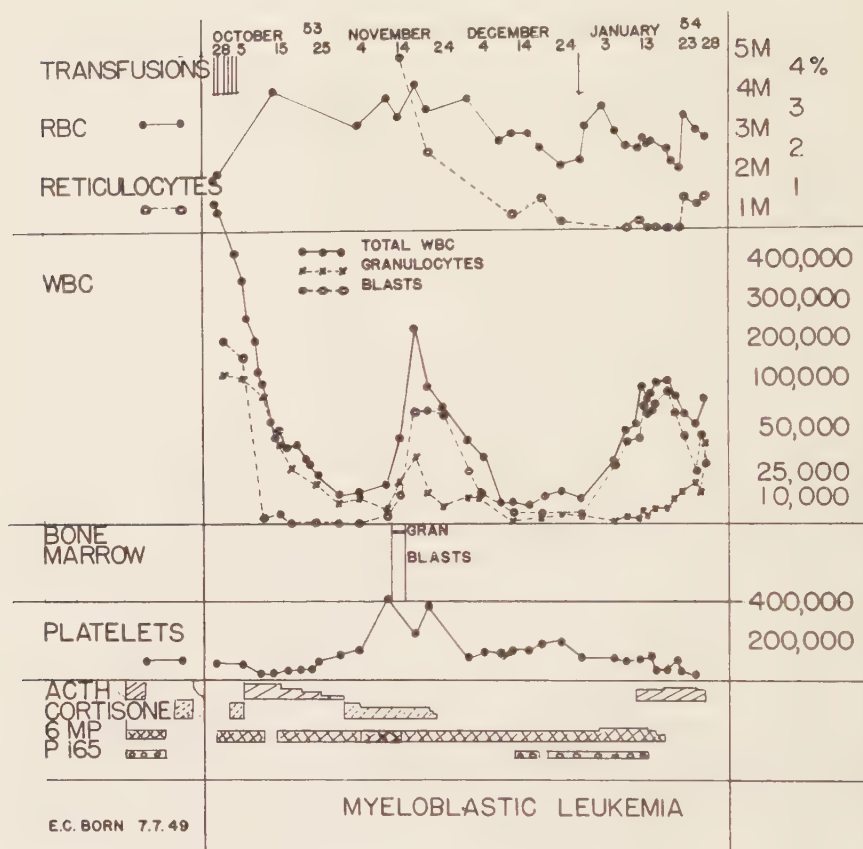


FIGURE 4

A diagnosis of leukemia was established in this 13-year-old patient five months prior to his first visit to our clinic. A remission had been obtained at another hospital in December with Aminopterin and ACTH therapy. ACTH had been given at biweekly intervals between December and April.

In April, 1953, he was admitted to our clinic after a relapse had occurred. The physical signs and marrow pattern were those of a leukemia in a hypoplastic marrow phase. After another course of ACTH, the marrow responded with a stimulation of granulo- and erythropoiesis and platelet formation. This remission was sustained from May 10, 1953 to June 25, 1953, when a clinical and hematological relapse occurred, while on cortisone, 5 mgm. per day.

On June 25, 1953, when the WBC was 1500, 6-mercaptopurine, 200 mgm. (2.7 mgm. per kg.) was started and continued for eight days; the dose was reduced to 100 mgm. for seven days. After these 15 days of therapy, the WBC had fallen to 2450, and the blasts to 5 per cent; the drug was withdrawn for five days and, since the leukocyte count did not drop further, it was resumed at a dose of 100 mgm. daily. The leukopenia persisted and, after 21 days, the

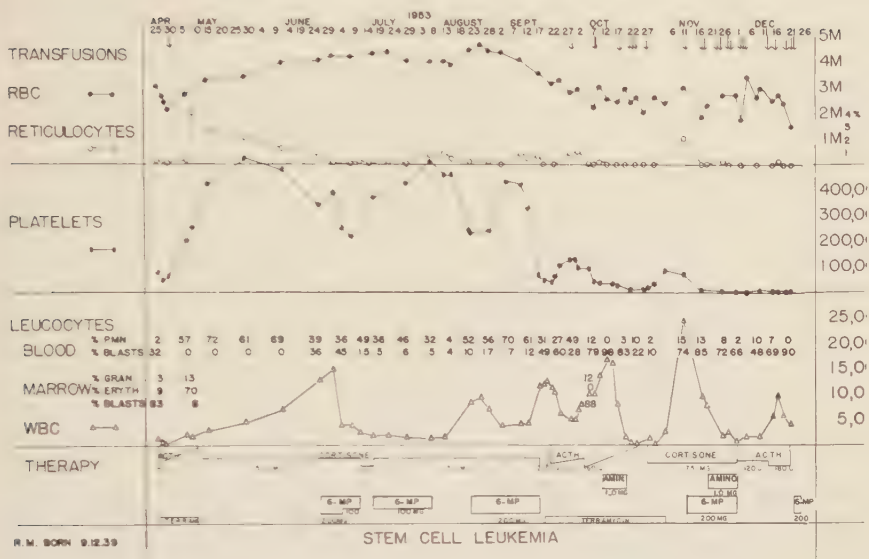


FIGURE 5

count had reached 1300, with only 2 to 4 per cent blasts in the blood; the platelet and RBC levels were good. Therapy was stopped for two weeks, and the WBC rose to 8500 and the blasts to 10 per cent. The drug was started again at 200 mgm. daily, and continued for three weeks, from August 21, 1953, to September 16, 1953. During this period, there was a transient fall in the blast count, but there was no improvement in the general hematological picture; the platelet count fell rapidly from 300,000 to 50,000, and the level of the RBC also fell. The patient remained ambulatory and in fairly good physical condition except for the signs of purpura.

At this point, the drug was stopped, and an attempt was made to control the relapse with ACTH without success. This treatment was followed by Aminopterin, 1.0 mgm. daily, which produced severe leukopenia of 550, without improvement in the platelet level. Under cortisone, 75 mgm. daily, the WBC rose again to 2400 with 74 per cent blasts, and the addition of 6-mercaptopurine combined with Aminopterin and cortisone was used. Although a leukopenia of 2800 resulted, the platelet level did not rise, the general condition deteriorated rapidly, and death was attributed to a generalized purpura and leukocytopenia.

### Results

The results obtained are summarized in TABLES 2 to 5.

In 6, or 27.3 per cent of the cases treated (see TABLE 2), clinical improvement was obtained which permitted ambulatory management; the children exhibited the general appearance of good health, and the hematological picture reflected a marrow remission, with a rise in the erythrocyte, reticulocyte, and platelet



TABLE 2  
LEUKEMIA IN CHILDREN  
TWENTY-TWO CASES TREATED WITH 6-MERCAPTOPYRINE  
*Remissions*

Type of leuk.	Hematological and clinical		Clinical only	Failures
	Good	Partial		
Stem myelo.....	2	5	2	6
Mono.....	4	0	0	
Leucosarc.....	0	2	0	1
Total.....	6 27.3%	7 31.8%	2 9.1%	7 31.8%

levels, and a return in normal granulocytes to the blood. The marrow remission was determined by: (1) a reduction in the percentage of blasts to 10 per cent or less; and (2) a regenerative pattern in granulo- and erythropoiesis. The early signs indicative of a marrow remission were usually apparent within 15 to 20 days after the drug was started and, in one case, remission began after 10 days of treatment. These six cases, which gave the best remission, tolerated the drug well and therapy was continued daily for from 4 to 24 weeks in this group. The duration of these good remissions varied from 4 to 14 weeks. In no instance was a second remission obtained after relapse had occurred, although the clinical status remained fairly good in several instances for a few weeks after marrow relapse had occurred. In two children, remissions were again obtained by a course of ACTH after relapse on 6-mercaptopurine.

In seven, or 31.8 per cent of the 22 cases, the remissions were classified as partial, since the clinical hematological status improved, but the marrow was not cleared of blasts. In these seven children, the improvement was considered to be sufficiently satisfactory to justify continuance of the drug for several weeks. The duration of treatment in the group varied from 7 to 12 weeks. All cases had proved resistant to ACTH or A-Methopterin.

In 2, or 9.1 per cent of the cases, the remissions were classified as clinical.

TABLE 3  
LEUKEMIA IN CHILDREN  
TWENTY-TWO CASES TREATED WITH 6-MERCAPTOPYRINE  
*Remissions Correlated with Duration of Therapy*

Remission	Treated < 3 wks.		Treated > 3 wks.		Total	
	Cases	%	Cases	%	Cases	%
Good.....	1	14.3	5	33.3	6	27.3
Partial.....	1	14.3	6	40.0	7	31.8
Clinical.....	1	14.3	1	6.7	2	9.1
Failures.....	4	57.1	3	20.0	7	31.8
	7		15		22	

TABLE 4  
LEUKEMIA IN CHILDREN  
RELATIONSHIP OF THE INITIAL LEUCOCYTE COUNT TO REMISSIONS

W.B.C.	Good	Partial	Clinical only	Failures
100,000	* *			* * *
50,000		* *		
25,000	*	*		
10,000	*			
5,000	*	* * **	* *	* *

TABLE 5  
LEUKEMIA IN CHILDREN  
COMPARISON OF REMISSIONS OBTAINED WITH 6-MERCAPTOPYRINE ACTH AND THE FOLIC  
ACID ANTAGONISTS

Therapy	Number of Cases	Remission rate	
		Cases	%
F.A. Antag.....	33	15	55.5
ACTH.....	37	27	75.6
6-MP.....	22	6	31.8
treated >3 weeks .....	15	5	33.3

These children were treated only in the late stage of the disease, and were resistant to both hormone and F. A. antagonist therapy. The clinical status improved, and the leukocyte counts remained at a leucopenic range for 8 to 12 weeks respectively. However, the blood and marrow were never cleared of blasts.

In seven, or 31.8 per cent of the cases, no benefit was obtained from the drug. Three cases were treated for as long as three to six and one-half weeks without benefit, but in the remaining four cases, the drug was withdrawn after six to ten days, because of severe leukopenia.

In TABLE 3, the remissions are correlated with the duration of drug therapy. It is evident that the remission rate varied directly with the duration of therapy. Of the six good remissions obtained, five, or 33 per cent, were treated 4 to 24 weeks; of the seven partial remissions, six were treated 7 to 12 weeks; and of 7 failures, four, or 57.1 per cent were treated for only six to eleven days.

No correlation could be established between the duration of disease before

therapy and the remission rate, since only 4 of the 18 cases had not been treated previously with other antileukemic agents. However, two of these four cases responded with good remissions: the one was a monoblastic type, and the other a myeloblastic type. The remaining two cases were therapeutic failures, and both were leukemias of the stem-cell type.

No correlation was demonstrated between the degree of leukocytosis and the remission rate. In TABLE 4, it is seen that, in 11 of the children, the pre-treatment leukocyte level was above 10,000, and below this level in the other 50 per cent of the cases. Of the six cases experiencing good remissions, four had an initial leukocyte count above 25,000, but so did four of the seven failures. Although it was true that a sharp fall occurred in the cases with hyperleukocytosis, after three to ten days of treatment in most instances, this fall did not guarantee a remission. Moreover, the most prolonged remissions were obtained in the two cases having a low initial leukocyte count.

In TABLE 5, a comparison is given of remissions obtained in other small-case series which were treated by the same investigator, with the F. A. antagonist drugs and ACTH. It is seen that the remission rate, for the 15 cases which were treated with 6-mercaptopurine for more than three weeks is not as favorable as that obtained with either of the other two agents. These figures are misleading, however, as our series of cases treated with 6-mercaptopurine is heavily weighted with cases in the late stage of their illness, while this description is not true of the series treated with either of the other agents. In the Aminopterin series, this compound was the first chemotherapeutic drug to be tested in our center, and all of the cases were of recent onset. In the ACTH series quoted above, less than one fourth had been treated previously with Aminopterin. In the four cases we have treated of recent onset, however, good remission were obtained only in two cases, and these cases were not prolonged.

### *Summary*

(1) The result of treatment of 22 cases of leukemia in children with 6-mercaptopurine is reported.

(2) The case series included 19 cases of acute leukemia of the following types: stem-cell, 13 cases; myeloblastic, 2 cases; monoblastic, 4 cases. The three other cases were of the leukosarcoma group.

(3) The drug was administered orally, in doses of 2.5 mgm. per day in one dose.

(4) Fifteen cases were treated from three weeks to six months in this group; five good and seven partial remissions were obtained.

(5) Seven cases were treated less than three weeks; one case responded with a good remission after 10 days of therapy, two cases gave partial remissions, and four cases were not benefited.

(6) In this small series, the acute monoblastic cases responded better than did the stem-cell type of leukemia to 6-mercaptopurine therapy.

(7) Of this series, only two children are now living. Withdrawal of the drug in the other 20 cases was eventually required owing to (1) a severe leukopenic effect; (2) drug resistance; or (3) failure to respond.

(8) Satisfactory remissions of three months or more were obtained in 6 of 22 cases treated.

## 6-MERCAPTOPURINE THERAPY IN NEOPLASTIC DISEASE\*

By R. Wayne Rundles and John A. Crago

*Department of Medicine, Duke University School of Medicine, and the Hematology Laboratory, Duke Hospital, Durham, N. C.*

In 1942, Hitchings and his collaborators began a study of the ability of pyrimidine derivatives to serve as precursors for, or to modify, nucleic acid synthesis.<sup>1</sup> Their findings now appear to have important implications in reference to cancer chemotherapy. Although, in earlier investigations, it appeared that preformed purine and pyrimidine bases were utilized in nucleic acid synthesis only by bacteria, later studies showed that adenine and 2,6-diaminopurine were incorporated into nucleic acid purine in mammalian tissues. Thymine was found to have a therapeutic effect comparable to that of folic acid in human nutritional megaloblastic anemia. The discovery of the anti-leukemic properties of folic acid antagonists made it appear more likely that purine and pyrimidine antimetabolites might serve as chemotherapeutic agents. In a screening program, using a spectrum of mouse tumors and mouse leukemia, several compounds showed oncolytic activity.<sup>2, 3</sup> The most promising of these compounds was 6-mercaptopurine.

The clinical effects of 6-mercaptopurine were studied by Burchenal *et al.*<sup>4</sup> Remissions were produced in a substantial number of patients afflicted with acute leukemia and chronic granulocytic leukemia. Therapeutic benefits were observed in some who had become refractory to other agents. Toxic reactions were infrequent.

The present studies were undertaken to provide a broader clinical evaluation of 6-mercaptopurine. We have studied its effect in 20 patients with acute leukemia, in two with multiple myeloma, and in one with undifferentiated metastatic tumor (TABLE 1). In most patients, other agents had been used previously until they had become ineffective or were found to be without benefit. The chemical was given at the rate of 2 to 4 mgm./kg. daily, initially in two or more divided doses, and continued in all cases until there was evidence of either therapeutic effect or a depression in leukocyte count. Maintenance therapy was usually given subsequently to those who were benefited. The following cases illustrate a partial and a "complete" remission observed in two patients having acute leukemia.

*Case 1.* T. C. J. D-67451. This 5½-month-old white boy was admitted to Duke Hospital on June 25, 1953. His illness of two weeks' duration had had a rapid onset with loose stools, abdominal swelling, and purpura. On physical examination he was fretful, pale, and febrile. His weight was 7 kg. Petechial hemorrhages were present over the skin and mucous membranes. The superficial lymph nodes were slightly enlarged. Examination of the blood showed a hemoglobin concentration of 7.4 gm. per cent and a white blood count of 66,000 with the following differential count: neutrophils 8 per cent, stabs 7 per cent, metamyelocytes 24 per cent. Bone marrow, aspirated from the tibia,

\* 6-Mercaptopurine was furnished for these studies by Doctor George H. Hitchings, Wellcome Research Laboratories, Tuckahoe, N. Y.



TABLE 1  
EFFECT OF 6-MERCAPTUPURINE THERAPY IN 23 PATIENTS HAVING NEOPLASTIC DISEASE

	No. patients treated	Effect of 6-MP				Treatment period	
		Fall in WBC	Complete remission	Partial remission	No benefit	> 3 wks.	< 3 wks.
Acute (myeloblastic) leukemia							
(A) Children	8	8	3	2	3	6	2
(B) Adults	10	10	2	6	2	7	3
Chr. granulocytic leukemia c acute exacerbation	2	2		2		1	1
Multiple myeloma	2	2			2	1	1
Undifferentiated metastatic tumor	1	1			0		1

was found to be extremely cellular and contained a greatly increased number of immature granulocytes and many hemohistiocytes.

6-Mercaptopurine was administred as the initial therapy in this instance and continued throughout most of his illness (FIGURE 1). After three to four days, the leukocytosis began to subside. After 14 days, the child had become virtually afebrile and the white blood count normal. Two small blood transfusions were administered, and subsequently the hemoglobin level was well maintained. The purpura gradually cleared, but the platelet count remained somewhat low, and immature granulocytes persisted in the circulating blood.

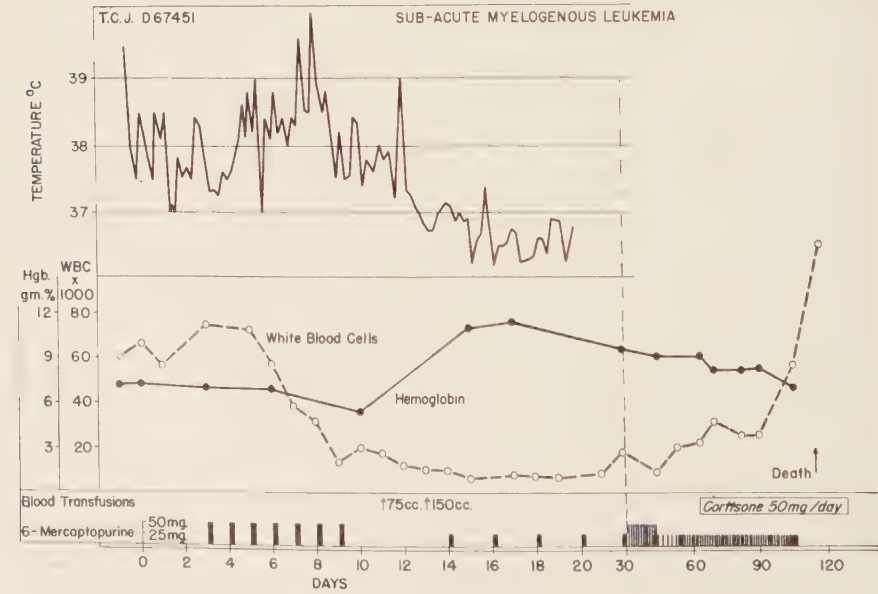


FIGURE 1

The child's general health remained good for a period of two months. Evidence of relapse then became apparent. In spite of an increased amount of 6-mercaptopurine and, eventually, the administration of cortisone, he died 19 weeks after the beginning of therapy.

*Case 2.* R. E. S. D-27081, a 32-year-old colored man, was admitted to Duke Hospital on July 7, 1953. For a period of about three months, he had had increasingly severe anorexia, weakness, and periodic chills and fever. On physical examination, he appeared weak and chronically ill. There was a moderate increase in tenderness over the sternum and ribs. The liver was somewhat enlarged. The spleen was not palpable. The hemoglobin concentration was 3.5 gm. per cent and the white cell count 78,000, with the following differential: neutrophils 1 per cent, lymphocytes 2 per cent, promyelocytes 42 per cent, and myeloblasts 55 per cent. The platelet count was 9,000. Bone marrow aspirated from the sternum contained sheets of myeloblasts.

For a period of 12 days, the patient was given blood transfusions and ACTH intravenously without signs of improvement (FIGURE 2). During this time, he developed a high fever, severe pain in the left upper quadrant of the abdo-

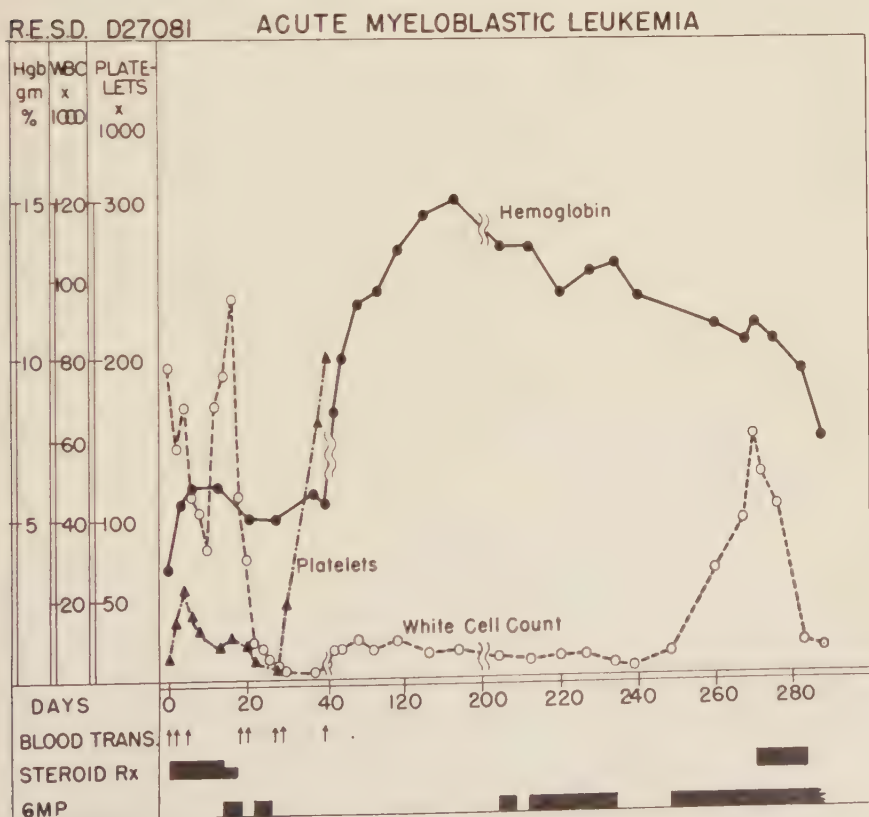


FIGURE 2

men, and extensive subconjunctival hemorrhages. His condition was finally regarded as critical.

6-Mercaptopurine therapy was begun at this time in a dose of 150 to 200 mgm. per day. Within three days, the white cell count fell precipitously. In spite of a reduced dose and, finally, suspension of 6-mercaptopurine therapy, the white count, 17 days after the chemical was first given, fell to a low of 900. During this period, the patient's symptoms abated rapidly. He was soon able to return home and resume work. On follow-up visits, his hematologic status became apparently normal.

Six months after the beginning of 6-mercaptopurine therapy, myeloblasts began to reappear in the peripheral blood. The administration of the anti-metabolite was resumed, but the patient slowly became worse. The white cell count rose to 60,000, a large majority of them being early granulocytes. The hemoglobin concentration fell to 10.5 gm. per cent. Platelets became moderately reduced in number.

The patient was readmitted to the hospital and given steroid therapy for nine days in addition to the 6-mercaptopurine. With the use of the two agents, the white count again fell to leukopenic levels, and his symptoms became less severe. During the following month, he was well enough to resume work, but anemia and abnormal leukocytes in the circulating blood persisted.

*Two multiple myeloma cases.* Two patients afflicted with multiple myeloma were treated with 6-mercaptopurine. One woman having multiple fractures had received no previous therapy. She excreted 3.5–5.0 gm. of Bence Jones protein in the urine daily. 6-Mercaptopurine was given for 26 days, during which time the white blood count became slightly depressed and the hemoglobin and hematocrit fell from 13.1 gm. and 42.0 per cent to 11.3 gm. and 35.6 per cent, respectively. There was no symptomatic improvement. The proteinuria remained unchanged. Urethane was then administered and clinical improvement was evident within one month. She subsequently became virtually asymptomatic. Her peripheral blood counts returned to normal, and the proteinuria fell to less than 0.5 gm. per day. A second patient with multiple myeloma had responded rather poorly to urethane and cortisone therapy over a period of 19 months. Following aggravation of his disease, he was given 6-mercaptopurine for a period of 18 days before he died without showing evidence of therapeutic benefit.

### Summary

The results of 6-mercaptopurine therapy in 8 children and 12 adults afflicted with acute leukemia, two of the latter having an acute exacerbation of chronic granulocytic leukemia, are summarized in TABLE 1. The effects in general were comparable to those of Burchenal *et al.* Partial remissions of the disease were observed in 10 patients (50 per cent); "complete" remissions in 5 (25 per cent); and no benefit in 5 (25 per cent). Remissions lasted from two to three weeks to over five months. Effects of the chemical other than on the bone marrow were rarely observed. Two patients developed hemorrhagic bullae in the skin and mucous membranes under full and possibly excessive therapeutic doses which subsided on withdrawal of the drug.

6-Mercaptopurine is a relatively nontoxic antimetabolite capable of producing temporary remissions in a substantial number of patients afflicted with acute myelogenous leukemia. Patients refractory to steroid and/or antifolic acid compounds may respond to the chemical, or additive benefits may be observed. 6-Mercaptopurine appears to act by a different metabolic mechanism than previously available therapeutic agents.

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# THE THERAPEUTIC EFFECT OF MERCAPTOPURINE IN A VARIETY OF HUMAN NEOPLASTIC DISEASES\*

By George A. Hyman, Alfred Gellhorn, and James A. Wolff

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This report summarizes the results of treatment of 87 patients with neoplastic disease utilizing 6-mercaptopurine (Purinethol, mercaptopurine). Particular attention will be directed toward the frequency of objective improvements in the acute leukemias and toward the toxic manifestations of drug therapy.

The clinical material studied in this therapeutic evaluation is tabulated in TABLE 1. The diagnosis was documented in every case by tissue biopsy or, in the leukemias, by bone marrow aspiration. The Purinethol was administered orally in daily doses of 2 mgm. per kilogram body weight. This dosage was gradually increased to as much as 7 mgm. per kilogram in patients who failed to respond to therapy. The duration of therapy is indicated in the summarizing tables in the succeeding section.

## *Results and Discussion*

(1) *Toxicity.* Nausea and vomiting was infrequent, occurring in but 9 of the 87 patients. It was, however, sufficiently severe in five to necessitate cessation of drug therapy. Although these individuals were not uremic, there was evidence of renal impairment, which may have contributed to the gastrointestinal manifestations.

A notable toxic reaction observed in two patients was drug fever. In both cases, this reaction of hypersensitivity developed after several weeks of treatment. The first patient, J. B., had reticulum cell sarcoma. The second patient, W. J., had acute leukemia. The syndrome of chills and fever up to 104° F. was reproduced in these patients on several occasions by the administration of a single dose of 25 mgm. of Purinethol. This result is illustrated in FIGURES 1 and 2. There was no associated skin rash, purpura, or change in peripheral blood counts.

Another probable manifestation of hypersensitivity was the occurrence of eosinophilia associated with protracted therapy which was noted in six cases. The eosinophiles reached 54 per cent of the peripheral leucocyte population in one case. The eosinophilia was not considered a contraindication to further therapy.

Hematologic depression attributable to Purinethol was the most frequent toxic finding. Leucopenia and/or thrombopenia led to eventual cessation of therapy in more than 50 per cent of the patients. This effect appeared most rapidly in the patients afflicted with advanced cancer, sometimes in less than 10 days, on a 2 mgm. per kilogram dose. In a few patients, especially in the children having acute leukemia, sepsis accompanied the leucopenia. Although a

\* These studies were supported in part by an institutional grant of the American Cancer Society, and in part by a grant of the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

TABLE 1  
SUMMARY OF 6-MP THERAPY  
87 Cases

Diagnosis	No.	Diagnosis	No.
Acute leukemia.....	12	Other cancer:.....	17
Acute leukemia (children).....	22	A) Bronchogenic Ca.....	5
Chronic myeloid leukemia.....	1	B) Renal Ca.....	2
Reticulum cell sarcoma.....	15	C) Ovarian Ca.....	2
Lymphosarcoma and Hodgkins.....	5	D) Gastric Ca.....	1
Multiple myeloma.....	15	E) Pancreatic Ca.....	1
		F) Rectosigmoid Ca.....	1
		G) Breast Ca.....	1
		H) Malignant melanoma.....	1
		I) Pinealoma.....	1
		J) Sympathicoblastoma.....	1
		K) Fibrosarcoma.....	1

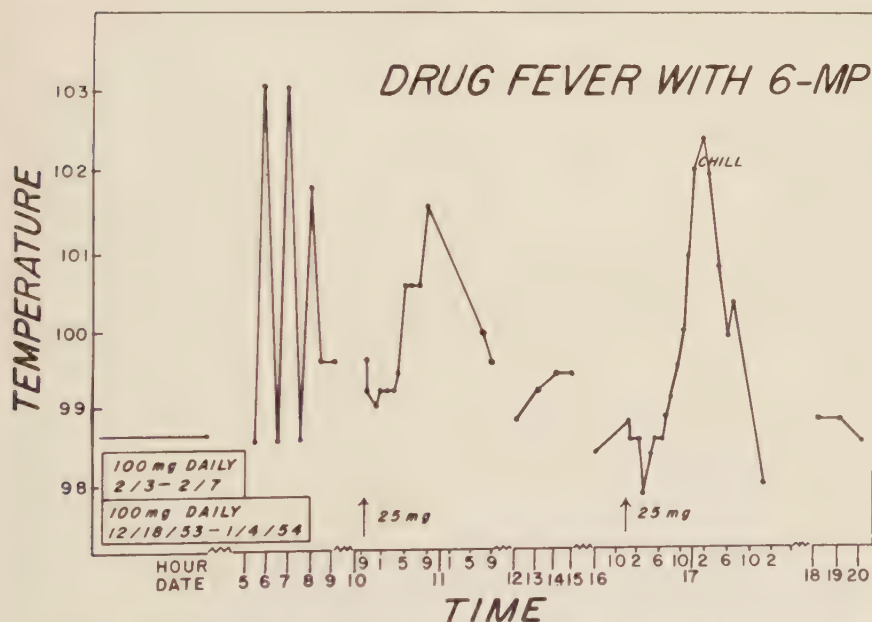


FIGURE 1. This 52-year-old man had generalized reticulum cell sarcoma. He received mercaptopurine, 100 mgm. daily for 17 days, from December 18 to January 4, and then stopped because of leucopenia. There was no other apparent biological effect of the compound, and the patient was afebrile. Purinethol was reinstituted at the same dosage in February, and after 5 days the patient had chills and spiking fever without hematologic depression. On two occasions, as indicated, 25 mgm. of mercaptopurine by mouth produced chills and fever. After the last test dose the patient remained afebrile until his death six weeks later, which was attributable to exsanguination from ulceration of gastric tumor infiltrations.

further fall in white cells and platelets might continue after Purinethol was stopped, spontaneous improvement was usually noted within 7 to 10 days. An initial fall in hemoglobin of 1 to 2 gm. per cent was often seen. Attempts to demonstrate a hemolytic mechanism as a basis for this drop were unsuccessful;

# **DRUG FEVER WITH 6-M.P. FEVER SECONDARY TO 6 M.P. THERAPY**

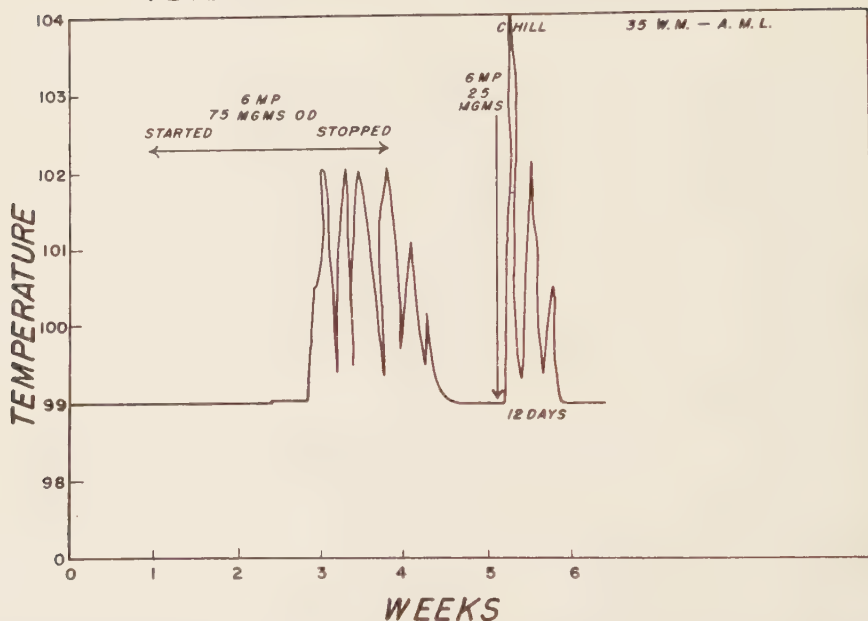


FIGURE 2. A 35-year-old white male having symptoms of purpura and fatigability for three weeks prior to diagnosis of acute myeloid leukemia on marrow aspiration. Initial therapy consisted of cortisone and repeated transfusions. After 14 days of mercaptopurine therapy chills and a high spiking fever appeared. One week after the drug was stopped, temperature became normal. A test dose of 25 mgm. of mercaptopurine 10 days later resulted in a similar episode of chills, fever, and malaise. The patient has been maintained on cortisone alone with occasional blood transfusions for eight weeks in a partial clinical remission.

the laboratory studies included both the conventional measurements for hemolysis and also examination of the disappearance rate of transfused red cells using the Ashby technique of differential agglutination.

(2) *Clinical effects.* TABLE 2A presents a summary of the results of therapy in acute leukemia of adults and children. A somewhat more detailed tabulation of pertinent facts about the individual patients is to be found in TABLES 3 and 4.

Twelve adults have been treated with mercaptopurine. Nine of the patients having this dyscrasia had received prior therapy. In two instances in which purpura was severe, cortisone was administered together with Purinethol. One of these patients had a hematological and clinical remission which lasted for three months. The cortisone was discontinued after the first week of therapy. In addition to the two good responses, one of which is still in a continuous remission after five months, there were two partial remissions and eight failures. Six of the latter patients died less than three weeks after treatment was started. There were no cases of acute monocytic leukemia in this series of patients.

Among the 22 children treated, there were seven good responses, six partial responses, and nine failures, three of the latter having been treated for less than three weeks. The majority of patients, after the initial few, were untreated by

TABLE 2  
SUMMARY OF 6-MP THERAPY  
87 Cases\*  
A. Leukemia (35 cases)

Diagnosis	No.	Results					Remarks
		Good	Partial	Clin. only	Failures		
					<3 wks.	>3 wks.	
Acute leukemia.....	12	2	2	—	6	2	
Acute leukemia (children).....	22	7	6	—	3	6	
Chronic myeloid leukemia.....	1	0	0			1	

B. Cancer Other than Leukemia (52 cases)

Diagnosis	No.	Results				Remarks
		Subj. & Obj. improve- ment	Subj. imp.	Failures		
				<3 wks.	>3 wks.	
Reticulum cell sarcoma	15	4	5	2	4	Remission 1 @ 14 mos. 3 @ 1 mo.
Lymphosarcoma and Hodgkins	5	0	2	2	1	
Multiple myeloma	15	2	1	6	6	Remission 1 @ 2 mos. 1 @ 6 mos. cont.
Other cancer	17	0	0	11	6	

\* This series includes patients from Delafield Hospital, Babies Hospital, and Presbyterian Hospital.

other agents prior to institution of mercaptopurine therapy. Because of the short time that Purinethol has been used clinically, the average duration of life in the responsive cases seems meaningless. It is of note that seven of the children lived for more than six months, the longest continuing remission to date being of nine months' duration.

One patient having late chronic myeloid leukemia had a good white count response without clinical improvement.

TABLE 2B summarizes our experience with 52 patients having neoplasms other than leukemia. Since the difficulty in classifying the lymphosarcomata is well known, Doctor Arthur Purdy Stout was kind enough to review all these sections for us. Reticulum cell sarcoma was encountered in 15 patients in a generalized form no longer suitable for radiotherapy. In one patient, a 14-month remission occurred with disappearance of fever, generalized glandular adenopathy, and hepatosplenomegaly. The patient returned to work. Relapse has recently recurred. Mercaptopurine therapy has been reinstituted after a 12-month hiatus, and there are indications of a transient response. Three other patients afflicted with this disease had shrinkage or disappearance of lymph nodes or tumor masses and other objective signs of improvement, but the ob-



TABLE 3  
RESULTS OF TREATMENT WITH 6-MP—ACUTE LEUKEMIA (ADULTS)

RESULTS OF TREATMENT WITH QUINACRIDINE						
Sex & age		Results				Remarks; cause for cessation
		Good	Partial	Failure		
				<3 wks.	>3 wks.	
F	34	x (1)			x	Death
M	41					(1) 3 mos. Remiss. (2) Refract.
F	38			x		Death
F	18			x		Death
M	55			x		Leucopenia, hemorrhage
F	71				x	Failure
M	70			x		Leucopenia, death
M	38		x			2 mos. remiss. cont.
M	21	x				5 mos. remiss. cont.
M	39		x			2 mos. remiss. cont.
M	35			x		Fever
M	65			x		Death, cerebral hem.
M—8						
F—4						
Tot. 12		2	2	6	2	

TABLE 4  
RESULTS OF TREATMENT WITH 6-MP—ACUTE LEUKEMIA (CHILDREN)

Sex & age	Results				Duration of treatment (days) &/or cause for cessation
	Good	Partial	Failure		
			<3 wks.	>3 wks.	
F 3 $\frac{3}{4}$	x				160 D. cont.
F 5				x	Leucopenia
M 4 $\frac{1}{2}$				x	Failure
F 3	x				215 D. cont.
M 6 $\frac{1}{2}$				x	Failure
F 8	x				246 D. leucopenia
M 3 $\frac{1}{4}$		x			211 D. hemorrhage
F 7 $\frac{1}{4}$	x				64 D. cont.
F 2		x			90 D. cont.
F 2 $\frac{1}{6}$				x	Transfer
M 5			x		Death
M 4 $\frac{3}{4}$		x			280 D cont.
M 1 $\frac{1}{2}$				x	Death
F 8 $\frac{1}{6}$		x			222 D. refractory
F 4 $\frac{1}{2}$	x				166 D. refractory
M 2 $\frac{1}{2}$	x				221 D. refractory
M 3 $\frac{1}{2}$	x				208 D. refractory
F 8 $\frac{1}{2}$			x		Leucopenia, sepsis
F 6 $\frac{1}{2}$				x	Leucopenia, sepsis
M 7		x			12 D. cont.
F 12		x			19 D. cont.
M 6			x		Leucopenia
M—10					
F—12					
Tot. 22	7	6	3	6	

jective improvement was shortlived, averaging one month. Although several other patients showed symptomatic improvement, this amelioration alone would not be justification for use of Purinethol, since other agents produce the same response. The evidence available suggests that, in generalized reticulum cell sarcoma, which is no longer amenable to radiotherapy, a trial on Purinethol is justifiable at least until the value of the drug is better defined.

No response was seen in five patients having other types of lymphosarcoma, or Hodgkin's disease. Results in 15 patients afflicted with multiple myeloma were disappointing, only two showing objective improvement for 2 and 6 months respectively.

Seventeen patients having a variety of solid tumors received Purinethol. Hematologic toxicity appeared with extreme rapidity in many instances. None of the patients showed improvement.

### *Summary*

In our experience, Purinethol has proved to be a useful therapeutic agent in the acute leukemias, especially in children. In a small number of patients afflicted with reticulum cell sarcoma, limited objective improvement has been observed, so that further investigation of the effect is warranted. In our hands, the agent appears to be of little value in multiple myeloma and of no value in lymphocytic lymphosarcoma, in Hodgkin's disease, or in any of the solid tumors studied to date. In addition to hematologic depression and gastrointestinal side effects, impressive drug fever was demonstrated in two instances, and eosinophilia, probably related to drug therapy, was noted in several patients.

## TREATMENT OF LEUKEMIA AND RELATED DISORDERS WITH 6-MERCAPTOPURINE

By F. H. Bethell and D. S. Thompson  
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Since February 1953, 48 patients afflicted with leukemia and allied disorders have received 6-mercaptopurine at the University of Michigan Hospital and at the Simpson Memorial Institute. Eight of the patients died within three weeks of the time of institution of therapy, most of them receiving the drug for less than one week. The remaining 40 serve as the clinical material for the analysis of our therapeutic results. It is recognized that for comparisons of antileukemia agents from the standpoint of survival, all cases should be included. However, such a comparison is not possible for our series because of previous or subsequent use of agents other than 6-mercaptopurine in many instances. It is our purpose to evaluate the nature of the response obtained with 6-mercaptopurine in those patients who received the drug alone for a sufficient time to permit observation of its effect.

Our practice is largely confined to adults, and in the series of 40 cases only five patients were under 15 years of age. It soon became evident that our better therapeutic responses were being obtained in younger adults, and that the most satisfactory dividing line appeared to be at 35. This is an arbitrary separation, of course, but one cannot help being impressed with the inability of the middle-aged and elderly patient having acute leukemia to restore any degree of normal hemopoiesis, even though leukemic cell proliferation is suppressed.

Factors which influence the responsiveness of a case of leukemia to specific medication, include, in addition to the age of the patient, the cell type, the proliferative activity, and the degree of dedifferentiation. Duration of the disease before institution of treatment bears a relation to the therapeutic response, but this variable cannot be controlled or readily defined, and it may be expected to affect more or less equally the several categories of acute leukemia.

The rapidly proliferating leukemias are classed as acute. The most undifferentiated type is the stem cell or hemocytoblastic. Those showing some cellular differentiation are the lymphocytic, granulocytic, and monocytic. Less rapidly proliferating forms of leukemia are classed as subacute. Most subacute leukemias are granulocytic, and this type only is represented in the present series. Chronic leukemias show variable rates of cell proliferation and, until they become advanced, exhibit a relatively high degree of cell differentiation. In addition to these forms there are the disseminated lymphocytic sarcoma and reticulum cell sarcoma, both of which may be leukemic. Employing the criteria for good and partial remissions suggested for the conference on 6-mercaptopurine on which this monograph is based, our results may be summarized as follows:

There were three cases of acute hemocytoblastic leukemia, all under 35 years of age. Two of these patients had good remissions, and one a partial remission.

There were four cases of acute lymphocytic leukemia. Of three patients under 35, two had good remissions and one a partial remission. One patient over 35 had a partial remission.

The series includes 10 cases of acute granulocytic leukemia. Of the seven patients under 35, two had good remissions, four had partial remissions, and in one there was no favorable response. Of the three patients 35 years of age or over, one had a partial remission and two had no responses.

A total of four cases of acute monocytic leukemia were treated. Two of these patients were under 35; the one had a partial remission and the other no remission. The two patients who were over 35 failed to respond to 6-mercaptopurine.

There were six cases of subacute granulocytic leukemia. Of the two patients under 35, the one had a partial remission and the other no remission. Four patients 35 or over included three who had partial remissions and one who had no remission.

Six patients having chronic granulocytic leukemia, all in the terminal acute stage of the disease, were treated with 6-mercaptopurine. Two were under 35, and four were 35 or older. All six patients had remissions which, from a hematologic standpoint, must be classed as partial, but clinically these remissions were good to excellent, and they lasted from a few weeks to several months.

The series includes six cases of disseminated lymphocytic sarcoma, three under and three over 35 years. Two of the younger group had good remissions and one a partial remission. Of those over 35, two had partial remissions and one no remission.

One patient over 35 years of age afflicted with wide-spread reticulum cell sarcoma obtained a partial remission while receiving 6-mercaptopurine.

*Discussion.* The number of patients in each category is small, so that interpretation of the data must be regarded as tentative. The results so far obtained with 6-mercaptopurine indicate that favorable responses may be obtained in a high percentage of children and of young adults having acute leukemia. The most favorable effects, in our experience, have been observed in patients suffering from acute hemocytoblastic and acute lymphocytic leukemia. Least beneficial results were obtained in monocytic leukemia. In all of our cases of acute leukemia under the age of 35, the percentage incidence of good remissions was 28.6, whereas the incidence of partial remissions was 33.3 per cent. Over the age of 35 there were no good remissions, and only 2 partial remissions occurred among the six patients treated.

In the less actively proliferative subacute leukemia of granulocytic type, no good remissions were obtained. Although patients having this form of the disease may be partially controlled and may have protracted illnesses, substantial hematologic and clinical improvement has not been observed in any of our cases.

6-Mercaptopurine is an exceedingly useful drug, in our experience, in the management of the late stage of chronic granulocytic leukemia when other forms of therapy are no longer effective. A sense of well-being is usually temporarily restored to such patients, and the extreme hypermetabolism and apprehensiveness of the terminal phase of this disease is largely averted.



The compound may be of considerable value in the treatment of disseminated lymphosarcoma and reticulum cell sarcoma.

No specific toxic effects, other than those on bone marrow, have been noted in association with 6-mercaptopurine therapy. Megaloblastoid transformation of erythropoiesis, such as may occur as a result of administration of folic acid antagonists, has rarely been observed after 6-mercaptopurine therapy. However, prolonged administration, for at least four or five weeks, is accompanied by a peculiar change in the morphology and staining reaction of the nucleated erythroid elements in the marrow. The early normoblasts show a loss of normal nuclear chromatin architecture which can best be described as karyolysis. The chromatin strands swell, lose their normal distinct ropelike pattern and become homogeneous opaque masses. Simultaneously there may be a great increase in the total number of nucleated erythrocytes.

*Conclusions.* Forty-eight patients afflicted with leukemia were treated with 6-mercaptopurine. The series included acute, subacute, and advanced chronic leukemia and disseminated malignant lymphoma. Evaluation of therapy is based on data obtained on 40 patients who were observed for at least three weeks.

Dosage of 6-mercaptopurine was within the range of 2.0 to 3.0 mgm. per kilo of body weight daily for initial therapy, and a total of 25 to 50 mgm. daily for maintenance in adult cases.

The results indicate that 6-mercaptopurine has a limited value in the treatment of acute leukemia, other than monocytic, in children and young adults. It is useful as palliation in the terminal stage of chronic granulocytic leukemia, and its administration may be temporarily beneficial in disseminated malignant lymphoma.

The combined or sequential use of 6-mercaptopurine with other antimetabolites and with cortisone and ACTH has, in general, improved the outlook of patients having acute leukemia.

# CLINICAL OBSERVATIONS OF THE TREATMENT OF LEUKEMIA AND ALLIED DISORDERS WITH 6-MERCAPTOPURINE

By James R. Fountain

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Within the past ten years a number of chemical agents have been discovered which produce temporary beneficial effects in patients afflicted with leukemia and allied disorders. In the majority of instances the discovery of these agents has resulted in one of three ways:

(a) The observation that a compound being investigated for other reasons has a depressant effect on hemopoietic tissues. Nitrogen mustard<sup>1, 2, 3</sup> falls into this group, as does urethane.<sup>4, 5</sup>

(b) The synthesis of compounds related chemically to the compounds already known to be effective. This explains the rationale behind the synthesis of triethylene melamine,<sup>6</sup> triethylene phosphoramide,<sup>7, 8</sup> and naphthyl-di-2-chloro-ethylamine (R.48).<sup>9, 10</sup> GT 41 (Myleran)<sup>11, 12</sup> and other sulphonic acid esters developed by Haddow also come into this group.

(c) The synthesis of analogues of nucleic acid precursors.

It is on the last group that so much attention is now being focussed. Undoubtedly the observation by Farber<sup>13</sup> that two folic acid conjugates, pteroyl-diglutamic acid and pteroyl-triglutamic acid, when given to children having acute leukemia resulted in acceleration of the leukemic process gave considerable impetus to research in this particular field. The sequel to this observation was the synthesis of the 4-amino analogues of folic acid, Aminopterin and A-Methopterin, which were found to suppress the growth of leukemic tissue and produce temporary remissions in some children having acute leukemia.<sup>14</sup> With the knowledge that folic acid was essential for the synthesis of nucleic acid, antagonists of other essential metabolites have been prepared and tested for antitumor activity. 2-6 Diaminopurine, an analogue of adenine, was shown by Burchenal<sup>15, 16</sup> to prolong the survival time of mice having transplanted leukemia. The same investigator later reported<sup>17</sup> remissions in five out of a series of 25 patients having acute leukemia treated with this compound. Compounds containing the 2,4-diaminopyrimidine moiety, including those with antimalarial activity<sup>18</sup> were shown to be competitive antagonists of folic acid in the growth of *Lactobacillus casei*<sup>19</sup> and, in animals, states indicative of folic acid deficiency was observed. Such findings suggested that such compounds might be effective in acute leukemia, and it has been shown<sup>20</sup> that one such pyrimidine analogue, 2,4-diamino-5 (3'4' dichlorophenyl)-6-methylpyrimidine, produced occasional remissions in children afflicted with this disease. Farber *et al.*<sup>21</sup> have shown that a related series of compounds, the dihydrotriazines, have the property of temporarily inhibiting the growth of the leukemic process in children.

Of the purine and pyrimidine derivatives discovered so far, the most effective in the treatment of acute leukemia is the analogue of adenine and hypoxanthine, 6-mercaptopurine (6-MP). This substance, known by the proprietary name

Purinethol, has been shown by Burchenal *et al.*<sup>22</sup> to produce good clinical and hematological remissions in 15 out of 45 children having the disease, and in another 10 patients partial remissions and clinical improvement were observed.

Since June 1953, 22 patients have been treated with 6-MP in Leeds. This figure includes 18 patients having leukemia, 2 having multiple myeloma, 1 having mycosis fungoides and 1 having erythroderma secondary to an underlying reticulosis. Of the patients afflicted with leukemia, nine had acute leukemia, seven chronic myeloid leukemia, and two chronic lymphatic leukemia.

*Treatment of leukemia with 6-mercaptopurine.* The plan of treatment was briefly as follows: patients having acute leukemia were unselected and none received other forms of chemotherapy such as folic acid antagonists, ACTH or cortisone prior to treatment with 6-MP. Blood transfusions and antibiotics were prescribed as the necessity arose. All patients received the initial course of therapy in hospital and clinical, and hematological examinations were performed either daily or on alternate days. Bone marrow examination was carried out at intervals in patients having acute leukemia. Those who improved sufficiently were later followed at the outpatient department and had blood counts at intervals of one or two weeks.

The dosage of 6-MP was based on that suggested by Burchenal, the initial dose being approximately 2.5 mgm./kg. body weight. The average 5-year-old child therefore started on 50 mgm., a ten-year-old, on 100 mgm., and an adult, on 150 to 200 mgm. per day. The same dosage scheme was used for both patients having acute and chronic leukemia.

### *Results of Treatment*

*Acute leukemia.* Of a total of nine patients, comprising four children and five adults, five showed a response to treatment with 6-MP. Two adults and one child achieved complete clinical and hematological remissions. The child, a boy aged three and one-half years, relapsed after a remission lasting three months (FIGURE 1), but developed a second remission which continued for four months. One adult male aged 46 years (FIGURE 2), having an acute myelomonocytic type of leukemia, remained in full remission for seven weeks, but failed to respond to treatment a second time. The other patient, a female aged 67 years, having acute lymphoblastic leukemia (aleukemic type) developed a complete remission which, to the present time, has lasted seven months.

The criteria on which a complete remission was based was strict and consisted of: (a) a disappearance of all clinical evidence of disease and a return of the patient to full activity; (b) a return of the peripheral blood picture to normal; and (c) a return of the bone marrow to apparent normality with a reduction of blast cells to below 10 per cent of the total cell count.

Two patients, one child and one adult, developed partial or incomplete remissions, *i.e.*, considerable clinical and hematological improvement but falling short of the criteria of a complete remission. Another child aged 10 years failed to show any hematological improvement after a seven-week period of treatment and observation. He was then given ACTH and, after four days, he developed a remission. Of the remaining three patients, two died within the

first week of treatment, the disease being extremely acute and associated with severe hemorrhage. The other, a male aged 76 years, failed to develop a remission after a prolonged course of treatment. He had an acute myelomonocytic leukemia, and died of broncho-pneumonia 18 days after treatment was discontinued. At death, the leucocyte count was 1,200 per cubic mm. The puzzling but extremely interesting finding was that post-mortem examination

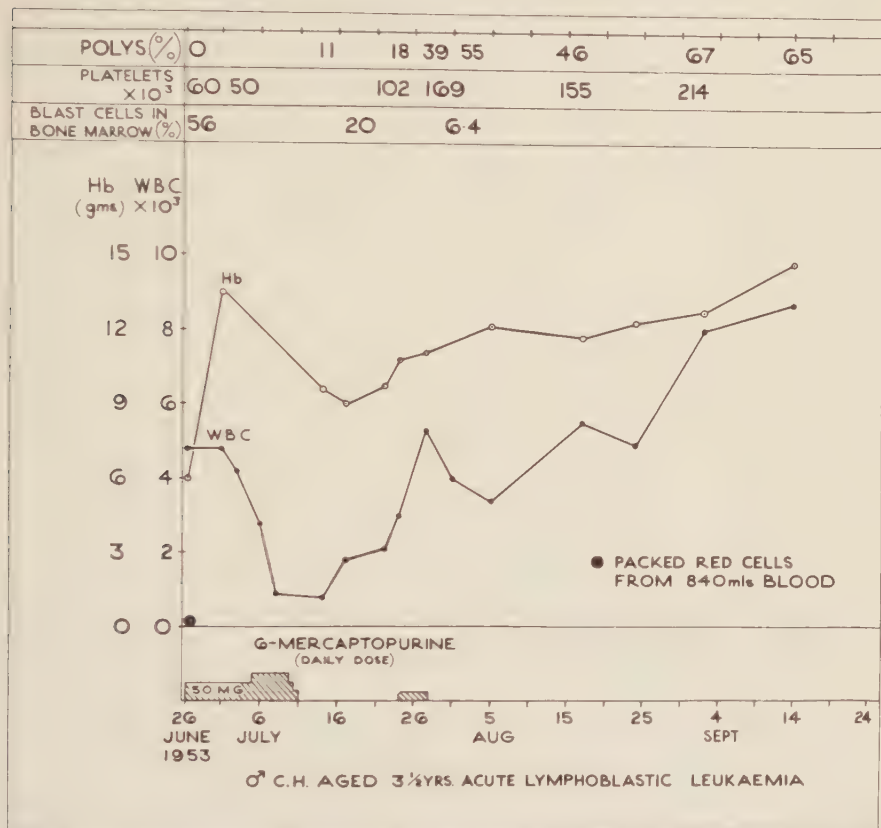


FIGURE 1. Acute lymphoblastic leukemia in a three and-one-half year-old male, treated with 6-MP, showing hematological remission.

failed to reveal any evidence of leukemia in the tissues examined, but histological evidence of Hodgkin's disease, apparently confined to lymph nodes in the mediastinum and abdomen, was observed. A possible explanation of this finding was considered to be that the primary lesion was Hodgkin's disease, which had subsequently evolved, incompletely, into a monocytic leukemia. The lack of obvious histological evidence of leukemia at autopsy can be suggested only as being attributable to the suppressive effect of 6-MP.

A delay of 10 to 28 days was observed in all patients before the drug became effective. A fall in the leucocyte count to a leucopenic level then occurred, at



which stage clinical evidence of the disease began to regress. There was one exception, a patient having aleukemic leukemia in whom no further reduction in the white cell count followed treatment, but who nevertheless developed a complete remission. Treatment was withheld as the leucocyte count fell below 3-4,000 per cubic mm., as experience had showed that the count continues to fall for several days after stopping treatment. During this leucopenic phase, which persisted up to three weeks, a reduction in size of the spleen, lymph nodes, and other manifestations of the disease was observed. This change was not necessarily, however, an indication of subsequent remission. In those patients developing remissions, hematological improvement also occurred. The bone

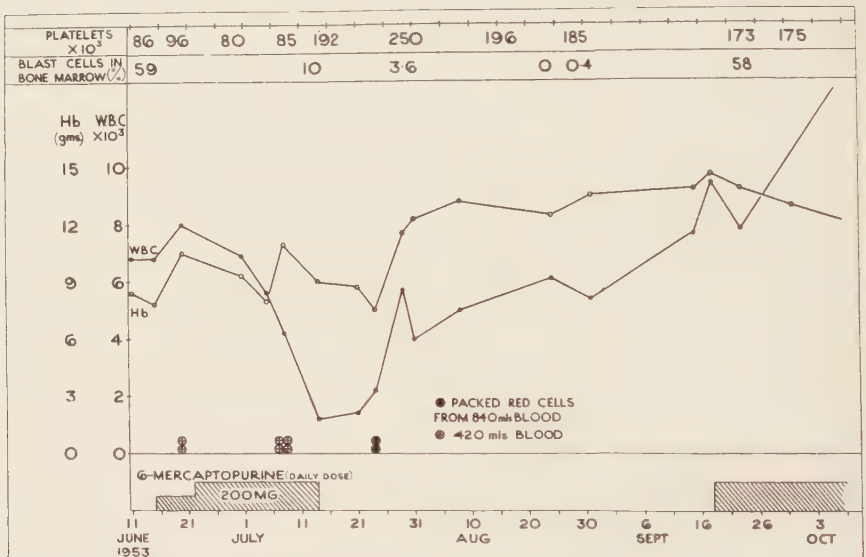


FIGURE 2. An adult male, aged 46 years having acute monocytic leukemia showing complete remission following treatment with 6-MP and then relapse.

marrow, apart from one instance, became hypocellular; the percentage of blast cells diminished, and this change was accompanied by increased erythroid and myeloid activity. A gradually increasing number of polymorphonuclear cells appeared in the peripheral blood, and a rise in the platelet count was an early feature. As the leucocyte count rose to normal levels, an apparently normal clinical and hematological state indicated that the patient had developed a complete remission. The persistence of leukemic cells in the bone marrow and peripheral blood in patients not showing evidence of such a remission was an indication to start therapy again, usually at a lower dose initially.

*Chronic myeloid leukemia.* It seems probable that one can expect clinical and hematological improvement in a high percentage of patients having chronic myeloid leukemia treated with 6-MP. Of seven patients at various stages of the disease treated with the drug, including one patient in the acute phase, a

response of varying degree was observed in six, the other patient dying of a cerebral thrombosis within a week of starting treatment.

The response to treatment was measured by: (1) clinical improvement, both subjective and objective; (2) a fall in the leucocyte count and a reduction of immature cells in the peripheral blood; (3) when anemia was present, by im-

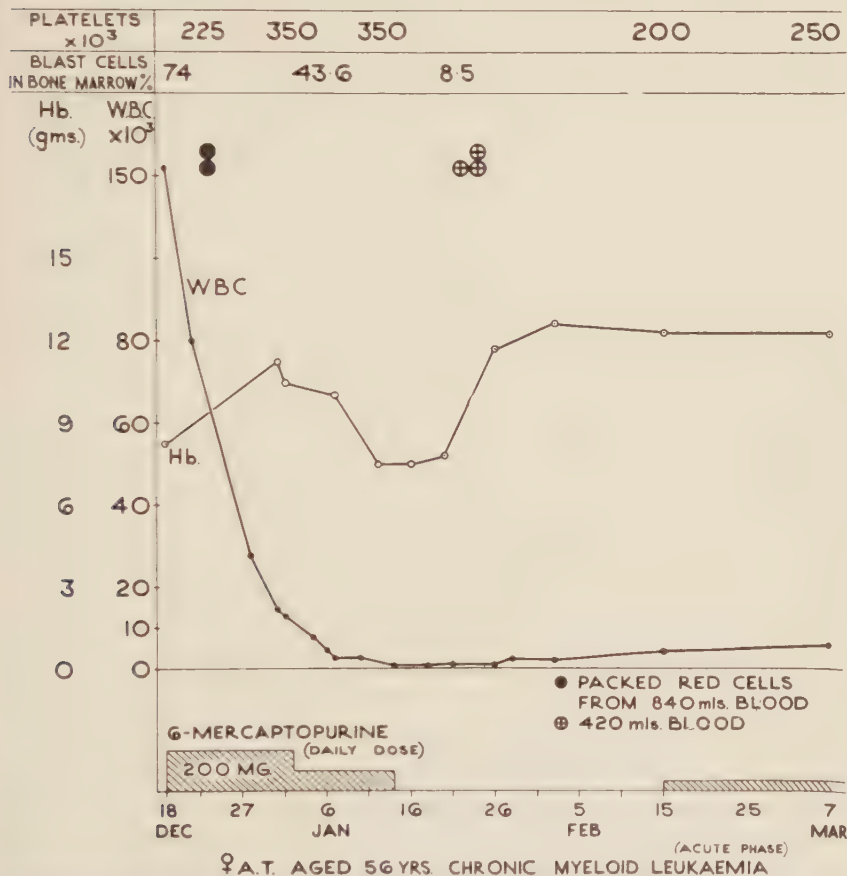


FIGURE 3. A 56 year-old female afflicted with chronic myeloid leukemia in the acute stage, showing hematological remission following treatment with 6-MP.

provement in erythropoiesis, and in the case of a patient in the acute stage, a reduction in the percentage of blast cells in the bone marrow.

In all six patients a fall in the leucocyte count with diminution in size of the spleen, liver, and lymph nodes was observed and in three, all the criteria of a good response were fulfilled. One of the latter (FIGURE 3), a patient in the acute stage, developed a complete remission which, to the present, has lasted for three months. Of the remaining three patients, one, although showing hematological improvement, suffered from a chronic respiratory infection and

showed no symptomatic benefit. Two other patients having severe anemia showed clinical evidence of improvement, together with a fall in the leucocyte count, but no improvement of erythropoiesis and blood transfusions proved necessary.

A delay before treatment became effective, was seen in patients having acute leukemia and in those having chronic myeloid leukemia. A fall in the leucocyte count then occurred, and the spleen, liver, and lymph glands became smaller. Treatment was stopped as the white cells fell to a normal level. The count continued to fall for several days after withdrawal of the drug, occasionally to leucopenic levels. While no complications resulted from these pronounced reductions in the white cell count, it would seem advisable to stop treatment at least temporarily when the leucocyte count reaches 30,000 to 40,000 per cubic mm. By so doing, serious depression of hematopoiesis should be prevented. Experience has shown that, within a month of stopping treatment, the leucocyte count rose and clinical evidence of the disease tended to become more apparent. It therefore seems desirable, if the disease is to be satisfactorily controlled by 6-MP, that maintenance therapy should be employed and, as relapse occurs after the initial course of therapy, treatment should be reinstituted at a lower level until a satisfactory maintenance dose is obtained (FIGURE 4).

*Chronic lymphatic leukemia.* Two patients having chronic lymphatic leukemia failed to show any clinical or hematological evidence of improvement.

*Miscellaneous diseases.* Two patients having multiple myeloma had 200 mgm. of 6-MP daily for three and four weeks respectively. No clinical, biochemical, or hematological improvement occurred. Both patients had advanced disease, and died toward the end of the course of treatment.

Two patients afflicted with skin disease were given 100 mgm. of 6-MP daily, a lower dose being prescribed in the hope that improvement might result without serious depression of hematopoiesis. One patient having a reticulosis of the skin failed to improve after four weeks' therapy, but another having mycosis fungoides obtained considerable benefit after a similar period of treatment. Relapse occurred after five weeks, but improvement followed a second course of 6-MP. Thereafter the residual lesions were treated with X rays.

*Toxic manifestations.* One of the important properties of 6-MP is its low degree of toxicity. Apart from producing a depression of bone marrow function it has not resulted, in this series, in any definite toxic side effects. Vomiting has been a temporary symptom in some patients, but on no occasion was it definitely attributable to the drug. It was not necessary to interrupt treatment on this account, and the vomiting has always ceased. Ulceration of the mouth, which so commonly follows treatment with Aminopterin or A-Methopterin was not observed. Severe thrombocytopenia attributable to 6-MP was observed in only one patient having multiple myeloma, and in no instance did use of the drug result in hemorrhage. No evidence of megaloblastic erythropoiesis, occasionally seen following antifolic treatment, was observed.

*Conclusions.* The results obtained from a study of this series of patients support the findings of other workers in that 6-MP has been shown to produce

temporary remissions in a percentage of patients having acute leukemia, and that it has the ability to modify the course of chronic myeloid leukemia. The remission rate in children having acute leukemia probably closely follows that of the folic acid antagonists, but the advantage it holds over these compounds is its low incidences of toxic manifestations. However, as Burchenal<sup>22</sup> has pointed out, patients becoming resistant to A-Methopterin therapy may respond to 6-MP. This absence of cross resistance is of obvious practical importance, and it indicates that both compounds are necessary for the management of patients having acute leukemia at the present time. Similarly ACTH and cortisone still hold their place in treatment, and are the choice when the patient has severe hemorrhage.

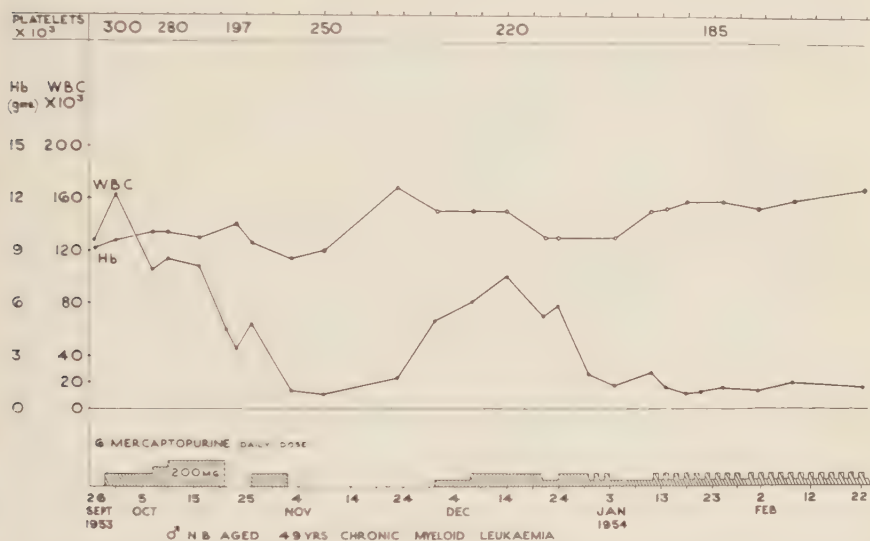


FIGURE 4. Chronic myeloid leukemia in a 49-year-old male showing a temporary response to a course of 6-MP and then continuing improvement with maintenance therapy.

With regard to the treatment of chronic myeloid leukemia with 6-MP or any other known chemical agent, it should be remembered that X-ray therapy, in the early stages of the disease, may result in prolonged remissions and is still probably the treatment of choice at the outset. Without an adequately controlled study one cannot say whether 6-MP at any stage of the disease has any advantage over radiotherapy, but our present knowledge suggests that it is the most effective therapeutic agent for those patients who have passed into an acute terminal stage.

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## THE USE OF 6-MERCAPTOPURINE IN ACUTE LEUKEMIA

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Our previous studies with acute leukemia indicated that Aminopterin (used preferably with ACTH or cortisone) was remarkably effective in acute lymphocytic leukemia of childhood, and that it was largely ineffective in the various types of acute leukemia in adults. The remissions in childhood leukemia by Aminopterin finally ceased, and complete refractoriness ensued. The acute granulocytic leukemia (myeloblast crisis) appearing as the end result of chronic granulocytic leukemia was always a terminal event for which only symptomatic therapy was available.

Our results with 6-mercaptopurine indicate a slight, though definite, improvement in this status. Only 15 cases have been studied: nine in adults, six in children. Results in this small series indicate: (1) that remissions can be obtained in childhood leukemia after complete refractoriness to Aminopterin therapy has occurred; (2) that long-sustained incomplete remissions can be obtained in the myeloblast crisis of chronic granulocytic leukemia; (3) that adult acute leukemia, whether granulocytic or lymphocytic, stands a fair chance of at least a partial remission. Also of interest is the fact that the platelet level is not greatly reduced and may even rise during the time of active therapy, in contradistinction to Aminopterin.

It is concluded that 6-mercaptopurine therapy represents an advance in the management of acute leukemia, indicating anew a certain degree of specificity of various chemical agents in various leukocytic proliferative syndromes, as well as the possibility of developing future chemotherapeutic agents that will attack various metabolic functions of the white cell.

# ROLE OF MERCAPTOPURINE IN THE TREATMENT OF LEUKEMIA AND RELATED DISEASES

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The effect of 6-mercaptopurine (6-MP) in the treatment of leukemia was initially reported by Burchenal and his associates.<sup>1</sup> This report presents results noted in the treatment of 61 patients who received 6-mercaptopurine (6-MP) as summarized in TABLE 1.

## *Methods of Study*

The initial dosage of 6-MP was 2.5 mgm./kg./day. This dosage was usually maintained for at least four weeks, depending upon the patient's response. If the leukocytes were depressed or if marked improvement occurred, the dosage was reduced to a quantity as low as 0.5 mgm./kg./day. In general, no untoward effect was noted following administration, except in two patients not included in this series, who discontinued the drug because of nausea. In general, other drugs were stopped during the use of 6-MP except in patients having acute leukemia and thrombocytopenia. These patients were given moderate doses of oral cortisone ranging from 25 to 75 mgm. daily in conjunction with the 6-MP.

## *Results*

*Acute leukemia in children (below 12 years).* Five of a group of 12 children developed remissions on treatment with 6-MP, as summarized in TABLE 1. The longest remission lasted for seven months, at which time the patient developed a relapse accompanied by fever, generalized malaise, and back pain. At present, this patient, whose data are summarized in FIGURE 1, has entered a partial remission on continued treatment with 6-MP. The data on two other patients who have developed remissions are shown in FIGURES 2 and 3. Two patients had partial benefit marked by transient improvement in the blood picture and apparent clinical response. These patients, however, have had a persistent anemia requiring periodic transfusions. In four cases, 6-MP had no beneficial effect, and was given for a brief period, until death, in one other patient.

*Acute leukemia in adults.* As summarized in TABLE 1, the results reveal that 12 of 35 patients showed partial improvement which was usually manifested clinically. Blood studies in a majority of these cases revealed decreases in the blast cells and the development of a leukopenia. There was little effect upon the red cell count, and the patients have required periodic transfusions. The severe thrombocytopenia, present in almost all the cases, was not improved by therapy.

One adult, who may be in an early stage of the disease, has been designated as developing a remission. The progress of this patient, however, has been followed for only three months.

Sixteen patients showed no improvement and six patients died before completing three weeks of 6-MP therapy.

TABLE 1  
SUMMARY OF TREATMENT OF 61 PATIENTS WITH LEUKEMIA AND RELATED DISEASES WITH 6-MERCAPTOPYRINE

Diagnosis	Number of cases	Remission	Partial remission	No effect	Died after <3 wks. of 6-MP	Living	Dead
Acute Leukemia							
Myeloblastic—children	8	4	2	2		7	1
Myeloblastic—adults	28	1	9	12	6	8	20
Monocytoid myeloblastic—children	1			1			1
Monocytoid myeloblastic—adults	6		2	4		2	4
Lymphoblastic—children	3	1		1	1	1	2
Lymphoblastic—adults	1		1				
Total—children	12	5	2	4	1	8	4
Total—adults	35	1	12	16	6	11	24
Chronic myelocytic leukemia (late stage)	10	0	6	3	1	6	4

Other Cases:

Chronic lymphocytic leukemia—1 case, no effect

Terminal polycythemia vera (myeloblastic phase)—1 case, no effect

Hodgkin's Disease—1 case, subjective improvement only

Chronic myelosis postsplenectomy—1 case, subjective improvement

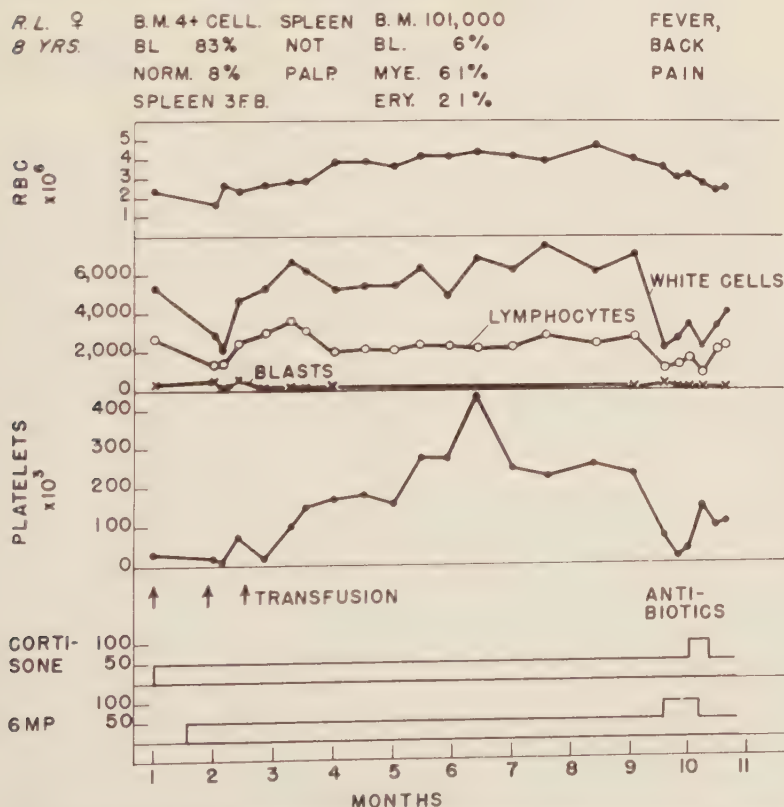


FIGURE 1. Chart of the effect of 6-MP and cortisone therapy in a child having myeloblastic leukemia. The patient had not received any previous medication.



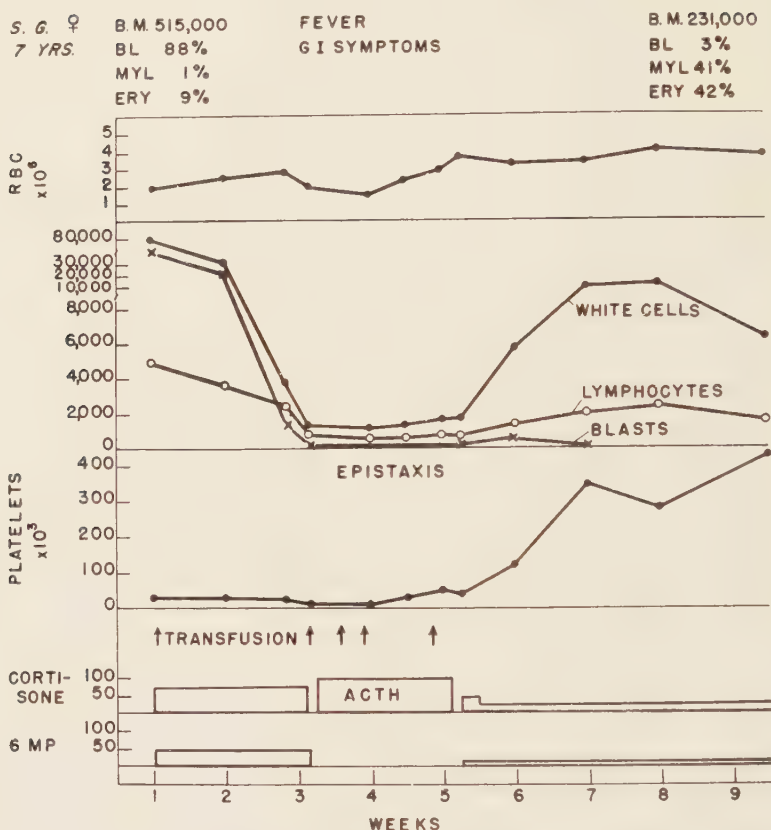


FIGURE 2. Chart showing a remission produced by combined 6-MP, cortisone and ACTH in a child having myeloblastic leukemia. No previous medication had been given.

Several of these cases were treated with other drugs before and after administration of 6-MP, as in the following examples:

Patient N. C., M, aged 27: triethylene melamine (TEM) no effect; triethylene phosphoramide (TEPA), no effect; cortisone, no effect; 6-MP and cortisone, no effect.

Patient B. E., M, aged 2: cortisone, no effect; A-Methopterin, slight effect; 6-MP and cortisone, slight effect.

Patient M. T., M, aged 48: TEPA, slight effect; 6-MP, no effect; Myleran, blood improvement, no clinical improvement.

In general, there was negligible difference among the three types of leukemia, that is, myeloblastic, monocytoid myeloblastic, and lymphoblastic leukemia, in their response to 6-MP. Remissions were produced in four of eight children having myeloblastic leukemia as compared with one of three having the lymphoblastic type. Among the adult patients, 33 per cent of both the myeloblastic and monocytoid types showed partial improvement. Untoward effects of 6-MP have been infrequent. Mouth ulcerations have been noted on one

B.M. ♂  
7 YRS.

B.M. 4+ CELL.  
BL 93%

B.M. 54,000  
BL. 2%  
MYL. 41%  
ERY. 49%

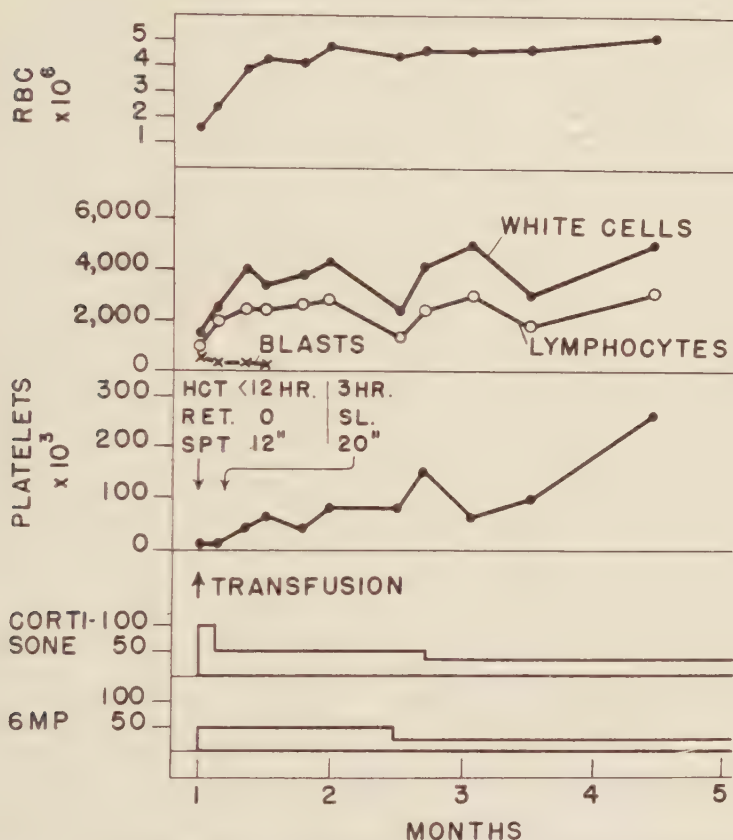


FIGURE 3. Remission in a child having myeloblastic leukemia. Patient is in remission at the present time.

occasion and may not have been specifically related to the drug. Six-MP does not appear to have depressed the platelets below pretreatment levels except in a few instances which were preceded by marked white-cell depression. One patient developed hyperuricemia.

*Chronic myelocytic leukemia.* TABLE 2 summarizes the results in 10 patients having the disease who were treated with 6-MP. All were in late stages of the disease. Duration prior to 6-MP therapy varied from 15 to 35 months; all patients had received other types of treatment previously, and all had become resistant to X ray, to arsenic, and to nitrogen-mustard derivatives. Splenomegaly was marked in all patients at the start of therapy.

One patient was treated for only two weeks. He was in a nearly terminal

TABLE 2  
TREATMENT OF CHRONIC MYELOCYTIC LEUKEMIA WITH 6-MERCAPTOPYRINE

Case	Sex	Age (yr)	Duration of disease prior to 6-MP (mos.)	Duration of 6-MP therapy (mos.)	Duration of remission (mos.)	Subsequent Course
B.A.	M	51	34	2	1	Remission on TEPA
H.B.	M	50	30	4	3	Died, homologous serum jaundice
R.F.	M	32	33	4	3	Died, Mar. '54 developed hyperuricemia on TEPA
M.G.	M	51	21	$\frac{1}{2}$	0	Died on 6-MP
F.G.	M	25	27	2	0	No response to Myleran, terminal
E.L.	F	53	15	6	5	Still in remission on 6-MP
H.R.	M	56	27	2	0	Developed hyperuricemia on 6-MP, died in uremia
B.W.	F	13	26	1	0	Remission on Myleran
S.W.	F	30	35	5	4	Remission with Myleran
M.W.	M	65	30	2	1	Still on 6-MP terminal

state at the time therapy was begun, having severe anemia, tremendous splenomegaly, high fever, myeloblastosis, and a serum uric acid level of 23.3 mgm. per cent. On 6-MP, his condition continued to deteriorate, and he expired after two weeks of treatment. The remaining nine patients were each treated for a minimum of four weeks. In six cases, moderate improvement was noted as to leukocytosis, splenomegaly, and anemia. This improvement lasted from one to five months. When it became apparent that relapse had occurred despite full doses of 6-MP, use of the drug was stopped, and another therapeutic agent was tried.

Three patients, treated for from four to eight weeks, showed no beneficial response. In two of these, the administration of 6-MP was stopped and other therapy instituted. The third patient (H. R.), whose condition had been progressing rapidly prior to treatment with 6-MP, was much improved after one month of therapy. His white blood count had fallen from 495,000 to 45,000, and the spleen had shrunk considerably. Despite continuing therapy, his white blood cell rose to 652,000 in the next three weeks, his hemoglobin fell, and the spleen enlarged rapidly. At that time he developed hyperuricemia, with uretal colic attributable to blockage by uric acid crystals. Despite supportive therapy and urological manipulations, he declined rapidly and died.

Two of the 10 patients having CML treated with 6-MP have died in close relation to this therapy. In one of these patients (M. G.), the drug was probably not responsible, while in the other (H. R.), hyperuricemia and uric acid blockage of the kidneys seemed to bear some relation to the drug.

Hyperuricemia occurred, in all, in three of these patients; in patient M. G. it antedated the use of 6-MP therapy, while in patient R. F. it occurred two weeks after the cessation of 6-MP, in close relation to injections of a nitrogen mustard derivative. Hyperuricemia as a complication of 6-MP treatment has occurred, therefore, only once in this series of 10 patients. This reaction represented the only serious untoward effect of the drug.

*Chronic lymphocytic leukemia.* No beneficial effect was noted in one patient having chronic lymphocytic leukemia who was treated with 6-MP.

*Other conditions.* One patient showing late manifestations of polycythemia vera, one having Hodgkin's disease, and one having chronic myelosis were treated with 6-MP. In none of these were there any objective changes noted in physical or laboratory findings. The patient with Hodgkin's disease (of 10 years' duration) felt stronger and had less fever during therapy than before it. The patient having chronic myelosis also felt stronger during treatment.

### Discussion

The results of this study are similar to those obtained by Burchenal *et al.*<sup>1</sup> Five of 12 children having acute leukemia developed remissions up to seven months in duration. One of 35 adults developed a remission which has lasted one month; 12 other adults revealed partial improvement either clinically, in the blood, or both. In the adult cases, however, there is no evidence that the drug specifically prolonged the life of the patient. The effect of moderate oral doses of cortisone, given in conjunction with 6-MP to a majority of our cases of acute leukemia is difficult to interpret, but it does not appear to have altered the response to 6-MP in comparing our results with those of Burchenal *et al.*<sup>1</sup>

Six of 10 patients in a late stage of chronic myelocytic leukemia were improved for periods of one to five months by the use of 6-MP. One patient developed hyperuricemia and ceased in uremia, secondary to blockage of both ureters by uric acid crystals.

### Summary

Sixty-one patients were treated with 6-MP. Five of 12 children having acute leukemia developed remissions lasting up to seven months. One of 35 adults having acute leukemia has developed a remission of one month's duration; 12 patients had partial improvement. Six of 10 patients in a late stage of chronic myelocytic leukemia were improved by 6-MP.

### References

1. BURCHENAL, J. H., M. L. MURPHY, R. R. ELLISON, M. P. SYKES, T. C. TAN, L. A. LEONE, D. A. KARNOFSKY, L. F. CRAVER, H. W. DARGEON, & C. P. RHODES. 1953. Clinical evaluation of a new antimetabolite, 6-mercaptopurine, in the treatment of leukemia and allied diseases. *Blood*. **8**: 965.



## REPORT ON 6-MERCAPTOPURINE

By V. P. Sydenstricker

*Medical College of Georgia, Augusta, Ga.*

Experience with 6-mercaptopurine (6-MP) in our clinic has been too limited to warrant more than a factual report of the results obtained. Only eight patients have received the drug, and of these only one had no other therapy. Two patients came under observation since the abstract for this conference was prepared.

Dosage followed the suggestions of Burchenal *et al.*, 2.5 mgm./kg./day for children and adults, though two adults were given 5 mgm./kg./day from the start.

The drug has been well tolerated by our patients. One developed nausea after two weeks on a 5 mgm./kg./day regimen, and one had a transient hematuria which probably was not attributable to the drug. No patient developed oral lesions or evidence of marrow injury that could not be attributed to the neoplastic disease under treatment.

### *Results*

*Acute leukemia.* Case No. 5, L. S. S. A six-and-one-half-year-old girl having acute lymphatic leukemia was seen on Dec. 1, 1953. At that time she was thin, pale with innumerable petechiae, there was generalized lymphadenopathy and marked enlargement of the spleen and liver. Hb. 5.5 gm., RBC 2,370,000, WBC 3,000 with PMN 2, lymph. 97 of which 40 were blasts, mon. 1, eos. 1. The platelet count was 10,000. She was given 250 cc. of fresh blood daily from December 4 to December 9, when Hb. was 14.8 gm., RBC 4,480,000, WBC 2,700 with 1 neutrophile and 99 lymphocytes on differential count. Platelets were 11,800. ACTHAR Gel was begun on this date, 80 units daily for five days, then 40 units for three days. No significant change in the blood picture occurred, and treatment with 6-MP was begun on December 19, 50 mgm. daily. By December 28 there was marked clinical improvement with disappearance of petechiae and regression in size of lymph nodes and liver and spleen, also there was great improvement in appetite and activity. A blood count done on that day showed 13.9 gm. Hb., 4,150 RBC, 2,500 WBC of which 48 were P.M.N., 52 lymphocytes with only 8 blasts. Improvement has been maintained to the present time, the most recent count showing 12.5 gm. Hb., 3,500,000 RBC, 2,250 WBC with PMN 66, lymph. 26, all mature, 5 eos., 3 bas.

Case No. 6, D. D. A 5-year-old boy having a history of suppurative mastoiditis and furunculosis of two months' duration came under observation on Jan. 26, 1954. The mastoids had been drained and curetted but failed to heal, and he had received repeated transfusions on account of progressive anemia. There was slight cervical adenopathy and moderate enlargement of the spleen. The blood showed 13.6 gm. Hb., 4,240,000 RBC, 9,150 WBC with PMN 1, lymph. 4, monocytes and monoblasts 81, and eosinophils 14. The bone marrow showed almost complete replacement of normal myeloid elements by monocytes, mono-

blasts, and reticulum cells, there was moderate eosinophilia and megakaryocytes, and platelets were adequate. A tentative diagnosis of acute monocytic leukemia was made, and therapy with 50 mg. of 6-MP daily was begun on January 30. To the present time there has been no marked change in his condition. In the absence of transfusions, hemoglobin and red count have fallen slightly. On March 26 the blood showed Hb. 10.5 gm., RBC 3,400,000, WBC 7,200, PMN 1, lymph. 8, monocytes and monoblasts 78, eos. 13. In spite of the free use of antibiotics and local measures, the mastoid wounds have not healed. The cause of eosinophilia is not evident. It seems possible that therapy has prevented a rapid downhill course in this child.

Case No. 7, L. W. A 48-year-old woman was seen on Mar. 15, 1954, on account of intractable anemia of two months duration requiring repeated transfusions. Except for pallor, the only physical findings were slight enlargement of the liver and spleen. The blood showed Hb. 9.2 gm., RBC 2,650,000, WBC 6,700, PMN 23, lymph. 11, myeloblasts (monocytoid) 66, platelets 112,000. Marrow showed myeloblastic proliferation, grade 4. Treatment was started on March 19 with 6-MP, 150 mgm. daily, and cortisone, 200 mgm. daily. Cortisone was continued for ten days at the original dose, then reduced 25 mgm. daily until discontinuance. This patient has shown no improvement, she requires frequent transfusions, and the most recent count, on April 17, was Hb. 11.3 gm., RBC 3,840,000, WBC 4,250, PMN 16, lymph. 18, myeloblasts 66. There has been gradual enlargement of the spleen, and recently the liver has become palpable.

*Chronic leukemia.* Case No. 1, L. J. A 32-year-old man had suffered from recurrent upper respiratory infections for two years, and in November 1952 was found to have myelocytic leukemia. It was stated that the leukocyte count was over 400,000 and hemoglobin 8 gm. He was treated with triethylenemelamine and radiation and transfusions, without improvement. He was referred to our clinic on Dec. 26, 1942, at which time he was pale and thin. Hb. 8 gm., RBC 2,800,000, WBC 480,000 with 14 neutrophiles, 10 metamyelocytes, 12 myelocytes, 60 myeloblasts and 4 lymphocytes. Platelets were abundant. On Jan. 2, 1953, he was given 7.5 millicuries of P32 without significant response. Later he received X-ray therapy to spleen and cancellous bones without improvement. On April 6, 1953, he was started on 6-MP, 150 mgm. daily. By April 20 there was great subjective improvement, and the blood showed 11 gm. Hb., 3,800,000 RBC, 38,000 WBC with 25 per cent PMN, 20 per cent metamyelocytes, 50 per cent myelocytes, 4 myeloblasts, and 1 lymphocyte. Improvement was maintained at approximately this level until May 20, 1953, when headache and nausea developed and the leukocyte count was found to have risen to 230,000 with a return to the exceedingly immature pattern found upon the first examination. Hemoglobin had fallen to 9.2 gm. and RBC to 3,000,000. He was hospitalized and given transfusions and other supportive therapy while 6-MP was increased to 300 mgm. daily. The leukocyte count rose rapidly and on June 1, 1953, was 503,000 with 16 per cent PMN, 5 per cent metamyelocytes, 16 per cent myelocytes, 60 per cent myeloblasts, and 3 per cent lymphocytes. Hemoglobin was 9.5 gm. and RBC 3,500,000. He expired early the next day. It is questionable whether this

patient acquired resistance to the drug or whether the dosage was inadequate during the earlier weeks of treatment. Autopsy showed the usual changes of chronic myelocytic leukemia.

Case No. 2, B.H. A 48-year-old woman was found to have chronic myelocytic leukemia in 1949. She was treated seriatim with X rays, urethane, arsenic, and occasional transfusions of blood over a period of about five years, during which time she remained able to work. About Feb. 1, 1954, she developed sudden paraplegia and severe pain in the legs. She was referred to the hospital where it was found that she was thin, pale, had general enlargement of the lymph nodes, massive enlargement of the liver and spleen, and a flaccid paraplegia and urinary retention. The blood showed 8.8 gm. hemoglobin, 29,00,000 RBC, 117,000 WBC with 48% PMN, 17 per cent metamyelocytes, 14 per cent myelocytes, 20 per cent myeloblasts, 1 per cent lymphocytes. She was given transfusions of blood, and treatment with 6-MP, 150 mgm. daily, was started. After three weeks she was much improved, pain was not a problem, though no recovery of motor function was evident. By March 1, hemoglobin was 11 Gm., RBC 3,400,000, WBC 18,400 with PMN 65 per cent, metamyelocytes 16 per cent, myelocytes 6 per cent, promyelocytes 10 per cent, lymphocytes 3 per cent. No significant change in the blood occurred before the patient expired on Mar. 16, 1954.

Case No. 3, M.J. A 29-year-old man, a Negro, had been found to have chronic myelocytic leukemia in 1951 and had been treated alternately with urethane and X rays with good results, so that he had continued at work. On Sept. 1, 1953, he was admitted to the hospital on account of weakness and fever. He was found to be severely anemic with great enlargement of the liver and spleen, hemoglobin was 6.8 Gm., RBC 2,700,000 WBC 20,000 with PMN 20 per cent, myelocytes 19 per cent, myeloblasts 60 per cent, lymphocytes 1 per cent. He was given transfusions of blood, and treatment with 6-MP was started on Sept. 6, 1953, 200 mgm. daily. After an initial rise in the leukocyte count to 67,000 on September 19, the count fell rapidly to a normal level of 6,700 with 74 per cent PMN, 6 myelocytes and 20 lymphocytes on September 27. Despite this excellent hematologic response, the patient died of pulmonary and renal infection resistant to current antibiotics on Sept. 28, 1953.

Case No. 8, D.E. A very obese colored woman, 33 years old, was seen Mar. 29, 1954. The spleen extended 10 cm. below the left costal border and mesially to the umbilicus. The blood showed 9.5 gm. Hb., 3,500,000 RBC, 187,000 WBC, with 40 per cent PMN, 35 per cent promyelocytes and myelocytes, 20 per cent myeloblasts, and 5 per cent lymphocytes. Platelets were 450,000. Treatment with 6-MP was started, 200 mgm. daily, on March 31. On April 7, following transfusions, Hb. was 14.3 gm., RBC 4,500,000, WBC 120,000 with 50 per cent PMN, 40 per cent promyelocytes and myelocytes, 6 per cent myeloblasts, and 4 per cent lymphocytes. On April 14 hemoglobin was 12.5 per cent, RBC 4,100,000, WBC 47,600 with 70 per cent PMN, 20 per cent promyelocytes and myelocytes, 5 per cent lymphocytes, 4 per cent monocytes, and 1 per cent eosinophils. Treatment with 6-MP was started,

200 mgm. daily, on March 31. On April 7, following transfusions, hemoglobin was 14.3 gm., RBC 4,500,000, WBC 120,000, with 50 per cent PMN, 40 per cent promyelocytes and myelocytes, 6 per cent myeloblasts, and 4 per cent lymphocytes. On April 14 hemoglobin was 12.5 per cent, RBC 4,100,000, WBC 47,600 with 70 per cent PMN, 20 per cent promyelocytes and myelocytes, 5 per cent lymphocytes, 4 per cent monocytes, and 1 per cent eosinophiles. The use of 6-MP was discontinued for four days and restarted at 100 mgm. daily. On April 21 there was no significant change in hemoglobin or red cells. Leukocytes were 24,000 with 80 per cent PMN, 8 per cent promyelocytes and myelocytes, 10 per cent lymphocytes, 1 per cent eosinophiles, and 1 per cent monocytes. The spleen was no longer palpable.

*Lymphosarcoma.* Case No. 4, W.H. A white man, 36 years old, was first seen Sept. 25, 1953. A diagnosis of lymphosarcoma had been made from node biopsy in June 1953, and treatment with X ray and triethylene melamine was carried out during July and August with no improvement. There was generalized lymph gland enlargement, much increase in the size of the liver and spleen, and evidence of lymphatic obstruction shown by massive edema of the genitalia and legs. The blood showed 11 gm. hemoglobin, 3,700,000 RBC, 5,500 WBC with 33 per cent PMN, 55 per cent lymphocytes, 8 monocytes, and 4 eosinophiles. The marrow showed marked lymphocytic infiltration but adequate numbers of normal myeloid elements. The platelet count was 72,000. Treatment with 6-MP was started on Sept. 28, 150 mgm. daily, and continued until October 15 when it was discontinued for seven days on account of nausea. Treatment was resumed on October 23 and continued until November 12. X-ray therapy was also used, and the patient received frequent transfusions. There was no subjective or objective improvement at any time, and the patient died Nov. 16, 1953. Autopsy showed generalized lymphosarcoma with no histologic changes attributable to therapy.



# RESULTS OBTAINED IN THE TREATMENT OF ACUTE LEUKEMIA AND LYMPHOSARCOMA WITH 6-MERCAPTOPURINE

By E. Clarence Rice  
*Children's Hospital, Washington, D. C.*

This report is based on our experience in the treatment of the following patients:

	Age years	Number patients
Acute lymphocytic or undifferentiated leukemia . . .	2 to 8	10
Acute myelocytic leukemia . . . . .	1	1
Acute myelocytic leukemia . . . . .	39	1
Lymphosarcoma . . . . .	8	1

All of the children had received either cortisone or A-Methopterin prior to treatment with 6-mercaptopurine. The shortest time a patient received this drug was 11 days, and the longest period of administration has been 10 months. The average duration of treatment with 6-mercaptopurine has been 13.6 weeks. The average dose has been 2.4 mgm./kg., with a minimum of 1.3 mgm. and a maximum of 3.6 mgm./kg.

The administration of 6-mercaptopurine has always been followed by a fall of the total number of leukocytes, with a subsequent increase when the drug was discontinued or the daily dose reduced in amount. In three patients, ulceration of the buccal mucosa was observed. No gastrointestinal symptoms attributable to 6-mercaptopurine or other toxic effects were noted by us.

While combinations of 6-mercaptopurine and other antileukemic drugs were used from time to time, we found no evidence that these combinations gave any better results than the individual drugs given alone, although one might expect combination therapy to give better results. One usually had the satisfaction of knowing that after the antifolics had ceased to be effective in the treatment of leukemia in a child, he could anticipate improvement in the patient's status following the administration of 6-mercaptopurine. On the basis of our experience with the antifolics and 6-mercaptopurine, we feel that there is little difference in the effects of the two groups of antagonists.

The best results were obtained in a group of eight children having acute leukemia, who were under six years of age. One experienced a complete remission lasting two weeks, and all except one obtained partial remissions of varying duration. An average of 23 days was required before definite evidence of a remission was observed.

Necropsy findings in children who had been treated with 6-mercaptopurine did not differ materially from those who had received other types of therapy, except that terminal bacterial invasion seemed more massive than previously.

The graphic records of two children who have been treated with 6-mercaptopurine are shown. FIGURE 1 shows a satisfactory response to the drug over a period of 28 weeks. This patient is now receiving 6-mercaptopurine in the tenth month of his leukemia and is doing well.

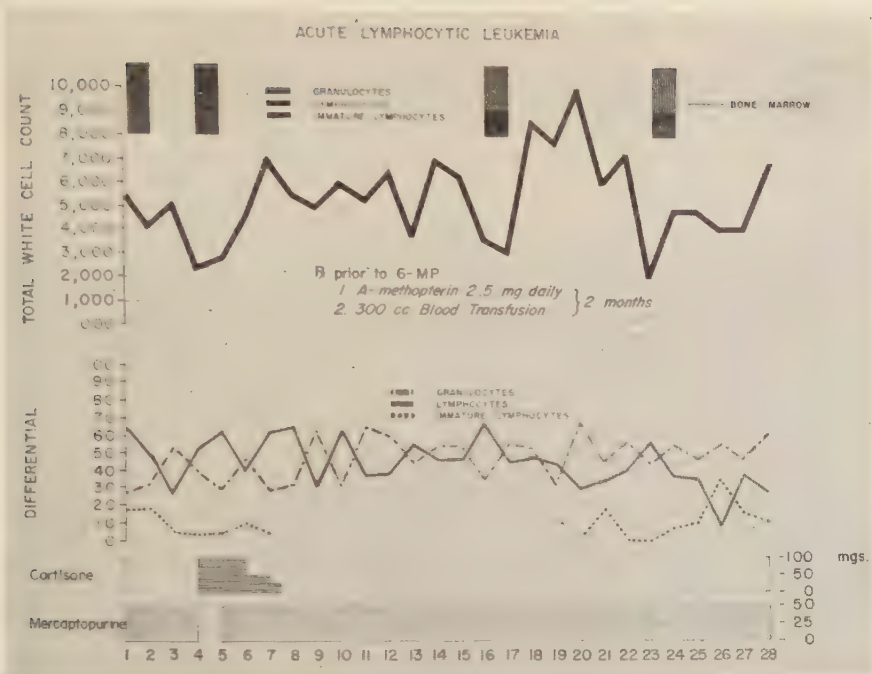


FIGURE 1. Acute lymphocytic leukemia of a male aged seven years. Shows portion of patient's record. 6-Mercaptopurine has been given for ten months.

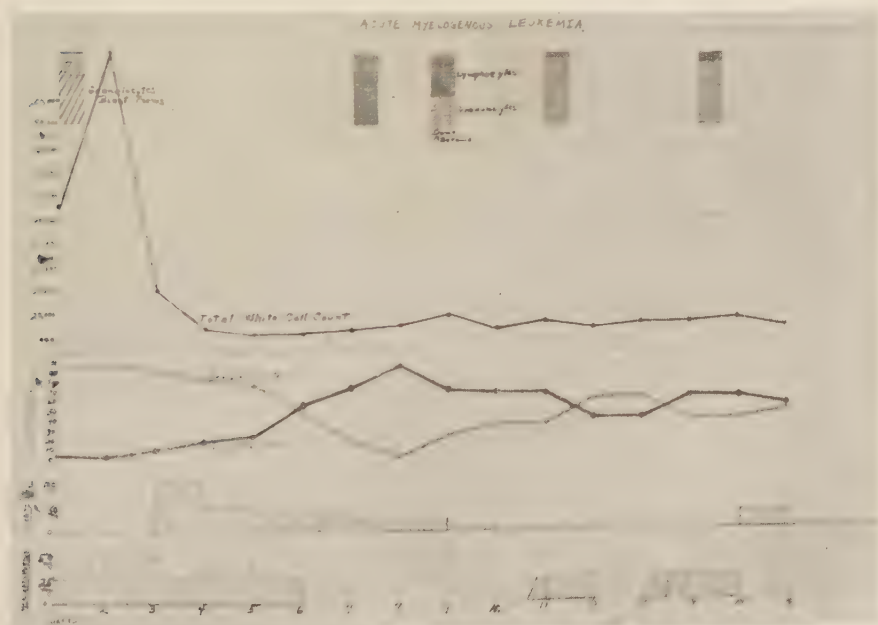


FIGURE 2. Acute myelocytic leukemia of a female aged one year. The duration of the disease was six months. Cortisone was given because of hemorrhagic complications.

AVERAGE DURATION OF SURVIVAL OF LEUKEMIA PATIENTS  
At The CHILDREN'S HOSPITAL, Washington, D. C.

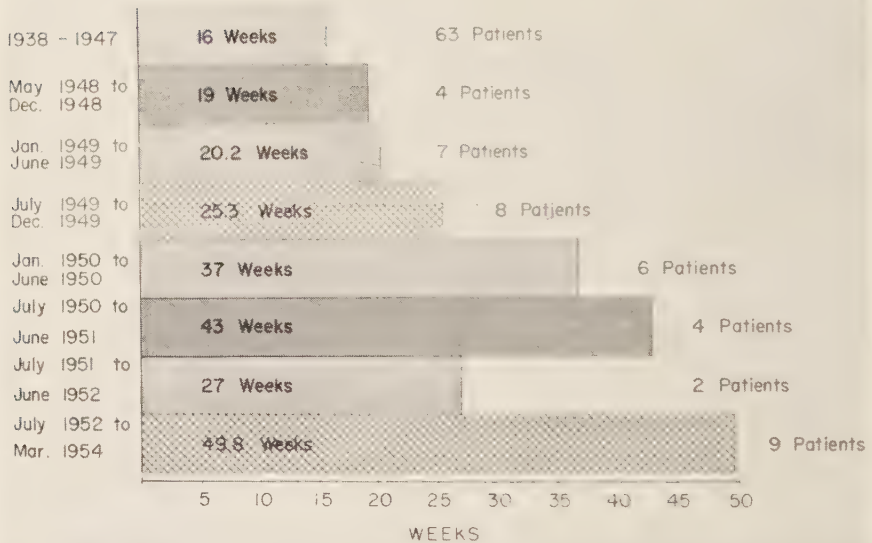


FIGURE 3

FIGURE 2 illustrates the treatment and findings in a one-year-old baby having acute myelocytic leukemia. The dose of 6-mercaptopurine averaged 1.3 mgm./kg.

6-Mercaptopurine represents a valuable addition to our therapeutic weapons for the treatment of leukemia in children. Its effects are similar to that of the antifolics. The availability of the antipurines, in addition to the antifolics, the steroids, and the antibiotics has made it possible to vary the attack to a greater degree, and to prolong still further the lives of our leukemic patients. Steroid therapy has the advantage not only of being antileukemic, but is of great value in combatting the hemorrhagic tendency seen in leukemia.

FIGURE 3 shows the duration of life from the time that the diagnosis of leukemia was made in 103 children at Children's Hospital. A 16-year period is represented. The average survival time at present is 49 weeks, compared with 16 weeks prior to the use of chemotherapy. The improvement in the results obtained has been most encouraging.

No benefit was observed following the treatment of one child having lymphosarcoma.

## 6-MERCAPTOPURINE THERAPY IN LEUKEMIA

By Joseph M. Hill and Jorge Lajous

*The J. K. and Susie L. Wadley Research Institute and Blood Bank; and Baylor University Hospital, Dallas, Texas*

Since April 29, 1953, 34 patients having leukemia have received 6-mercaptopurine (6-MP) therapy on the Wadley Institute hematologic service. For the most part, those receiving this drug had been treated previously with other agents, especially folic acid antagonists and ACTH or cortisone. In general, a remission was first sought with Aminopterin, or A-Methopterin alone (usually the latter). If no significant tendency toward remission was observed in four to six weeks, ACTH or cortisone was added in moderate dosage but discontinued as soon as remission was obtained. If no signs of remission appeared on this dual therapy, 6-MP was combined with the other two agents. In practice, only acute myelogenous leukemia required this triple combination.

Maintenance therapy was employed, once a remission was obtained with antifolics or 6-MP. ACTH and cortisone were used sparingly and reserved for later needs. However, in the presence of exceptionally low platelet counts or signs of hemorrhage, these agents were immediately employed, along with fresh blood or platelet concentrates. If coagulation studies showed deficiencies in prothrombin, proaccelarin or proconvertin, vitamin K<sub>1</sub> (Mephyton) was added to the transfused blood. Protamine sulfate was also used, particularly if heparinoid types of inhibitors could be demonstrated in the patient's blood.

Whenever possible, antibiotics were given in febrile episodes, according to sensitivity tests on cultured organisms employing combinations, as well as single antibiotics. Chloromycetin, Archomycin, terramycin, penicillin, and polymyxin were most frequently used with success. Empirical use of these agents singly and in combination was also freely tried in the more severe febrile states.

Other factors in the complete care of the patient were carefully observed, from nutritional requirements to the use of detergent-type antiseptics for all skin punctures.

Acutely ill hospital patients were followed with daily complete blood counts including platelets. Outpatients seeking remissions were checked with the same blood tests three times a week. Patients in remission, but on maintenance therapy, were similarly followed at intervals of one to four weeks.

The 6-MP was given according to individual response, in doses ranging from 0.4 mgm. to 5 mgm. per day, per kilo of body weight. Doses of more than 50 mgm. daily were usually divided. ACTH and cortisone or hydrocortisone were added to the therapy if no evidence of onset of remission was observed in four to six weeks. However, in acute myelogenous leukemia, combinations were resorted to earlier, or combination therapy used initially.

FIGURE 1 shows an analysis of 6-MP therapy in our series of 34 cases of leukemia. The remissions were graded as follows: one plus, clinical improvement only; two plus, definite hematologic improvement, such as reduction of blasts and increase of platelets; three plus, reduction of blasts below 5 per cent



## RESULTS OF 6-MP THERAPY IN 34 CASES OF LEUKEMIA

TYPE	C CHILD A ADULT	SEX	NO REMISSIONS	++OR+++ REMISSIONS	++++ REMISSIONS
LYMPHOCYTIC ACUTE	C	♂	1 <sup>f</sup>	2	3 <sup>s</sup>
	C	♀	—	—	4 <sup>s</sup>
MYELOCYTIC ACUTE	C	♂	—	1	—
	C	♀	—	1 <sup>s</sup>	—
	A	♂	5 <sup>fssc</sup>	2 <sup>s</sup>	3 <sup>fsc</sup>
	A	♀	2 <sup>sc</sup>	1 <sup>c</sup>	3 <sup>ssc</sup>
CHRONIC TURNED ACUTE	A	♂	3 <sup>sc</sup>	1 <sup>s</sup>	—
	A	♀	1 <sup>f</sup>	1	—

Each superscript indicates 1 case receiving additional therapy as follows:

f = amethopterin or aminopterin

s = ACTH and/or cortisone

c = f and s combined

All chronic leukemias turned acute were myelocytic

FIGURE 1

in peripheral blood, platelets increased over 100,000; four plus, essentially complete remission with no blasts in peripheral blood, platelets in normal range, and blasts below 30 per cent in marrow. Marrow examinations were part of the evaluation of most, but not all, of the remissions. In practice, two-plus and three-plus remissions were combined, since only two cases were three-plus. We were unable to satisfy ourselves that any case showed definite clinical improvement with no significant hematologic improvement, so no one-plus remission shows in the table.

All of the acute lymphocytic leukemias were in children, with 9 remissions out of 10 cases under 6-mercaptopurine therapy. Two cases lived 20 months and 13 months, respectively, after onset of the disease. The remaining eight cases still living averaged 14 months with the disease, while three of these cases exceeded 12 months' survival to this date. Two cases achieving remission (one boy, one girl) received ACTH or cortisone concomitantly. Each of the 10 cases had previously received courses of ACTH or cortisone, and A-Methopterin or Aminopterin. All 10 had prior remissions on folic acid antagonists.

As a group, the acute myelocytic leukemias responded less favorably to 6-MP with only 9 out of 16 achieving remissions, of which six were four-plus, or complete. Eight of the nine received combination therapy, as indicated. Some individual cases had excellent remissions, three surviving more than a year from onset. In this group, two cases received 6-MP therapy for less than three weeks.

The chronic leukemias in acute terminal phase (all myelocytic) obtained

fewer remissions than any other class, with only two partial remissions out of six cases. One of these subsequently has done well on urethane (four months to the present).

One case of myelocytic leukemia in the chronic-turned-acute group obtained a three-plus remission on 6-MP in the chronic phase. This case is indicated in FIGURE 1 as the single female obtaining a three-plus remission in this last group.

Among the patients having acute lymphocytic leukemia, the mean lengths of remissions and their standard errors were: for A-Methopterin and Aminopterin,  $34.6 \pm 7.47$  weeks; for 6-MP,  $7.6 \pm 1.61$  weeks. The difference is significant at the  $P = 0.01$  level. However, it is doubtful whether one could conclude, on such evidence, that folic acid antagonists are superior to 6-MP under comparable conditions. Most of the 6-MP remissions in our cases are still continuing at this date. Moreover, even if this were not the case, the effects of order of administration and stage of the disease could possibly alter the results. Remissions, as defined in this particular comparison, included only the interval during which the maximum benefit was derived from therapy.

In the case of acute myelocytic leukemia, the mean lengths of remission were: for A-Methopterin,  $\pm 13.21$  weeks; for 6-MP,  $\pm 8.35$  weeks. Comparison of these figures means even less, since some of the longest remissions were obtained with combined therapy.

FIGURE 2 illustrates the response of a four-year-old white female who had had a previous six-month remission on A-Methopterin. The patient was hospitalized having respiratory infection, pharyngitis, and a fever of  $101^{\circ}$ . Hepatomegaly, severe splenomegaly, and ascites were present; leukocyte count 2700, lymphocytes 96 per cent, hemoglobin 9.9 grams, and platelets 6,980. The child appeared moribund when 6-MP was started. Five days after ad-

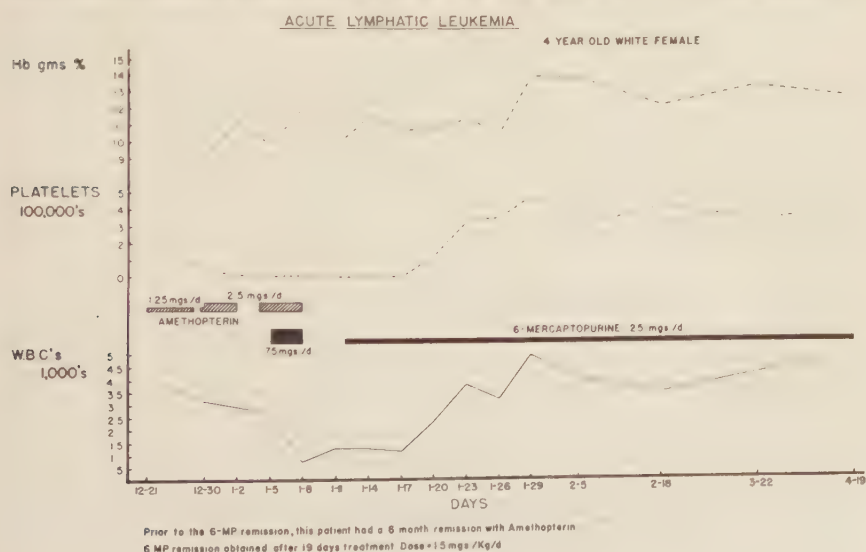


FIGURE 2

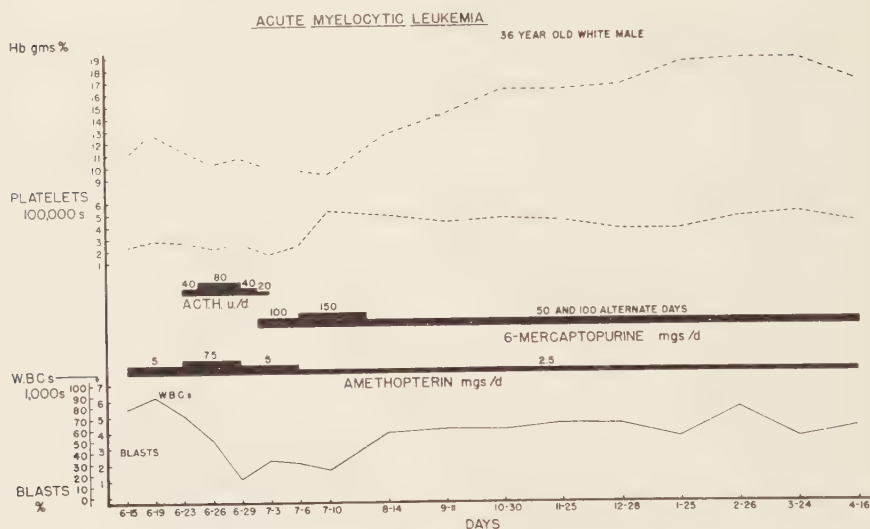


FIGURE 3

mission, fever was at  $103.6^{\circ}$ , and leukocyte count 600. On the ninth hospital day, the patient was improving, and went into remission after 19 days of treatment. The leukocyte count was normal, with 58 per cent lymphocytes, no hepatomegaly, and the spleen just palpable. The remission is continuing, with normal bone marrow after three months.

FIGURE 3 illustrates the best response in acute myelocytic leukemia of this series. This 36-year-old white male felt weak after an attack of "influenza" in May 1953. He required seven transfusions in three weeks. A diagnosis of acute myelogenous leukemia was made, and A-Methopterin therapy started on June 15, 1953. ACTH was added on June 22, and 6-MP on July 1, 1953. Myeloblasts dropped from 48 per cent to 0 in peripheral blood, and the bone marrow appeared practically normal in appearance, with only 5 per cent myeloblasts, as compared to 95 per cent in the initial marrow specimen. A recent examination made on April 16, 1954, showed that the marrow is still essentially normal.

### Discussion

The drug, 6-mercaptopurine, appeared to be very effective in the over-all percentage of remissions obtained in all types of acute leukemia observed in this series. It was most effective in acute lymphatic leukemia, all of which cases in this group were children. Compared with A-Methopterin and Aminopterin, there was a difference of only one case which responded to a combination of A-Methopterin and ACTH, but did not respond to 6-MP. As used in our series (following prior remissions with other agents), the remissions appeared somewhat shorter than with A-Methopterin and Aminopterin, but the data did not warrant the conclusion of any superiority under comparable conditions of trial.

In the acute lymphatic leukemias, no correlation of result with height of initial count was observed, since all but one case responded. In the acute myelocytic types, no remissions were obtained in four cases with initial leukocyte counts below 5,000. Out of the group with initial leukocyte counts in the 5,000 to 25,000 range, there were five remissions out of seven cases. In the 25,000 to 100,000 leukocyte group, there were four remissions out of six cases.

The youngest patient to obtain a remission had acute myelocytic leukemia at birth. The oldest patient, likewise myelocytic, was 76 years old. Both had only two-plus remissions.

In using the drug, it was noted that there was relatively little depressing effect on platelets, and a slightly greater depressing effect on leukocytes, compared to other agents. In some cases somewhat resistant to therapy, it was possible to obtain a remission by protecting the patient with antibiotics and continuing the 6-MP, in spite of leukocyte counts below 1000.

6-Mercaptopurine is a useful and most welcome addition to the agents which can be used in rotation, or in combination, to increase the survival time of patients afflicted with leukemia. Perhaps even more important are the new avenues opened up in the study of cell metabolism.



## 6-MERCAPTOPURINE IN ACUTE LEUKEMIA

By Eugene L. Lozner

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About three years ago, we reached an arbitrary decision, to the effect that all patients having acute leukemia, regardless of the age of the patient or the apparent cell type, would henceforth be treated first with hormones, usually ACTH, and that, during the second week of therapy, antifolic agents would be added. After a remission was obtained, if such were indeed to be the case, one of the agents was discontinued. More frequently it was the hormones, but the other agent has been maintained continuously.

With the advent of 6-mercaptopurine, in the above regimen, this new agent has been substituted for the antifolic drug in a few patients, beginning in March 1953. In only one patient was this pattern varied, and in this patient the initial therapy was 6-mercaptopurine and A-Methopterin. In this patient, however, after a good remission was obtained, a subsequent relapse was treated in the above manner. In a few of the relapses, all three agents were employed simultaneously.

As stated in the abstract, our series is so small as to permit only impressions. No conclusions are possible. Eight patients have been under direct observation, and the chart of one patient from Utica has been reviewed. The dosage of 6-mercaptopurine was usually 2.5 mgm./kg. or slightly less. It was never greater. Six patients were children and three adults. Of the children, three had good remissions, one a partial remission, and there were two failures. One of these failures is doing "well" with the other therapeutic agents.

Among the three adults, there was one good remission, one partial remission, and one failure. The good remission was in a 16-year-old boy.

Since 6-mercaptopurine was added to our regimen in March 1953, five patients (four children, one adult) have expired. Four are still living (two children, two adults). Only one is now on 6-mercaptopurine. This is the 16-year-

TABLE 1  
DURATION OF LIFE IN PATIENTS WITH ACUTE LEUKEMIA TREATED WITH  
6-MERCAPTOPURINE AND OTHER AGENTS

	Months from first symptom	Months from diagnosis
Child No. 1 (L).....	30	29
Child No. 2 (D).....	12	11
Child No. 3 (D).....	10	9
Child No. 4 (D).....	9	5
Child No. 5 (L).....	5	4
Child No. 6 (D).....	2½	2
Adult No. 1 (L).....	13	12
Adult No. 2 (L).....	4	3
Adult No. 3 (D).....	2½	1½

L—Still living April, 1954.  
D—Dead.

old boy to whom we have already referred. In the patients in whom 6-mercaptopurine was intentionally discontinued before death, the usual reason was severe leukopenia with evidence of marrow hypoplasia.

Less frequent was discontinuance because of resistance to this agent.

In *all* nine patients, both living and dead, in whom this agent was employed (never singly as already mentioned) the average length of life from the onset of the first apparent symptoms attributable to leukemia was 9.9 months to date. The usual interval between symptoms and definitive diagnosis was one month (TABLE 1).

In summary, we have the impression that 6-mercaptopurine is somewhat less effective than antifolic agents in acute leukemia in combination with hormonal agents, but may represent a significant addition to the roster of effective therapeutic agents.

# CLINICAL EXPERIENCE USING 6-MERCAPTOPURINE IN CHILDHOOD LEUKEMIA

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During the 13-month period since March 1953 that 6-mercaptopurine has been available to us, the drug has been employed as part of the therapy of 11 children having acute stem-cell leukemia. The diagnosis was established in all patients by bone marrow examination immediately prior to 6-mercaptopurine therapy.

At onset of 6-mercaptopurine therapy, the ages of the patients ranged from two to six years, and the total duration of their symptoms prior to therapy varied from two weeks to eleven months. The antimetabolite therapy was not discontinued during periods of remission, and was supported by vigorous general medical care. Their progress was evaluated by history, repeated physical examinations, and counts of peripheral blood. Our criteria for a hematologic remission were: clinical well-being, absence of visceral and lymph node enlargement, together with a normal hematologic picture of the peripheral blood. Bone marrow aspiration for confirmation of a hematologic remission was not done in all cases.

## *Results of Therapy*

Four of these 11 patients received 6-mercaptopurine after having become resistant to Aminopterin therapy. A single clinical and hematologic remission as defined above was produced in each of these four patients, lasting from two to nine months. With the exception of the child whose remission lasted nine months, who received 5 mgm./kg. for the first two weeks of therapy, all patients received an initial daily dose of 2.5 mgm./kg. of 6-mercaptopurine. All were given maintenance therapy during their periods of remission, the dosage consisting of 2.5 mgm./kg./day, or 1.2 mgm./kg./day if leukopenia were present. All have shown recurrence of their leukemia. One patient is now receiving cortisone. Three patients were given a second course of approximately 3 to 4 mgm./kg./day. In no instance was a second remission produced, and three patients have subsequently expired. After the initial 6-mercaptopurine therapy all had received a short course of cortisone before the second trial of 6-mercaptopurine therapy. Two autopsies were performed, and extensive generalized leukemic infiltrations were demonstrated. One child had developed multiple liver abscesses which yielded coagulase positive hemolytic *Staphylococcus aureus* on culture.

One patient had become resistant to both Aminopterin and cortisone prior to 6-mercaptopurine therapy. This child received 2.5 mgm./kg. of 6-mercaptopurine for only 11 days, and his leukocyte count fell from 15,000 to 700 per cubic mm. The drug was discontinued because of epistaxis, and he died two days later. No autopsy was obtained.

The second group of patients receiving 6-mercaptopurine had not yet become resistant to another drug.

One child was given 6-mercaptopurine alone as his initial therapy. He developed a partial remission, lasting about one month, midway during a three month period, ending in complete relapse. He was subsequently treated with Aminopterin and cortisone and, finally, with a second trial of 6-mercaptopurine. He received 3 to 4 mgm. kg. of 6-mercaptopurine for three days, with no apparent effect, dying in full relapse. An autopsy was performed and showed generalized leukemic cellular infiltrations, gastrointestinal bleeding not associated with degenerative mucosal changes, and multiple *Staphylococcal* abscesses of liver.

Four children have been given a combination of both 6-mercaptopurine and Aminopterin as their initial therapy. One developed symptoms of an intracerebral hemorrhage after seven days of therapy and later expired.

The remainder are still in clinical and hematologic remissions lasting four, one month, and one month respectively. The first has been receiving 25 mgm. day of 6-mercaptopurine and .25 mgm. of Aminopterin daily. The latter are receiving 50 mgm. 6-mercaptopurine daily (2.5 mgm./kg.) and .5 mgm. Aminopterin per day when free of buccal mucosal ulcerations.

A single patient was given both cortisone and 6-mercaptopurine as her initial therapy. A partial clinical and hematologic remission was produced in 13 days. The cortisone was discontinued after eight days and the 6-mercaptopurine continued. Except for a leukopenia, her peripheral blood picture showed no evidences of leukemic change. Despite this condition, her enlarged viscera never receded completely. Her leukocyte count rose to 21,000 during the early phases of clinical pertussis. One month later she began showing thrombopenia, leukopenia, and anemia, necessitating repeated transfusions. A bone marrow aspiration was done two months later and showed complete relapse.

### *Dosage*

In general, all children were given 2.5 mgm./kg./day of 6-mercaptopurine when receiving their first course of therapy. When the peripheral blood showed a total leukocyte count of 3000/cubic mm. or less, the dose was either decreased to 1.2 mgm./kg./day, or the drug was discontinued temporarily. By error, one of the first children to receive this drug was given 5 mgm./kg./day for two weeks. This patient developed prolonged leukopenia and thence progressed into a complete remission lasting nine months. When given concomitantly with Aminopterin, we arbitrarily reduced the amounts of both drugs to one half our usual therapeutic dose in one instance, but gave full doses of each to the last two. Four children received a second course of 6-mercaptopurine therapy after their leukemia had become resistant to all available agents. All children received 75 mgm./day or approximately 3 to 4 mgm./kg./day, but in no instance was this therapy effective in altering either the clinical or the hematologic picture.



*Toxicity*

Since the therapeutic effect of the antimetabolites is primarily that of marrow depression and interference with cellular maturation, we shall assume that the production of a hypoplastic state of the bone marrow is not considered to be a toxic effect for this discussion. We are more concerned with the side effects which prohibit continued therapy in full doses.

In our use of 6-mercaptopurine, the only side effect which has given us caution and, at times, reason to discontinue therapy, was buccal mucosal ulcerations. Patients receiving 6-mercaptopurine alone developed these lesions much less commonly than those who were receiving also the folic antagonist Aminopterin. Only two of seven patients receiving 6-mercaptopurine alone developed mouth ulcers, as compared to three of four who also were receiving Aminopterin therapy in addition. The fourth child was treated for only seven days, since she developed spontaneous intracranial hemorrhage and expired several days later.

Episodes of vomiting and abdominal pain were rare in the group receiving 6-mercaptopurine alone.

One child deserves special mention because of prolonged administration of a higher dose of 6-mercaptopurine than is usually recommended. This patient was treated initially with Aminopterin without effect, then with 6-mercaptopurine for five months. During this period he was clinically well for about three months, but was hematologically free of leukemic change only approximately two thirds of that time. Following this period he was treated with cortisone, with a brief period of remission. Then he was given a second course of 6-mercaptopurine. This time we elected to give 75 mgm./day (approximately 3 to 4 mgm./kg.). This dosage was administered for 24 days with no appreciable effect. By mistake, on the 24th day, he received 250 mgm. over a 24 hour period. The drug was then stopped. He developed no evidence of a bleeding tendency. Nine days later he was found to have a single area of buccal mucosal ulceration when readmitted to the hospital because of a nosebleed, fever, and abdominal pain. Physical examination showed generalized visceral and lymph node enlargement. His peripheral blood findings were: hemoglobin 8.9 gm./100 cc., WBC 13,250. Differential: 1 per cent neutrophils, remainder all mononuclear cells. Platelets 6,440/cubic mm. A blood culture yielded a hemolytic *Staphylococcus aureus*, coagulase positive. He was again given 6-mercaptopurine, in a dose of 50 mgm./day. His fever persisted despite intensive antibiotic therapy, and his abdominal pain increased. He expired in a shocklike state six days after admission, and an autopsy was performed. A generalized bleeding state was found in the skin, cutaneous serosal surfaces, lung, and focal areas of the intestinal submucosa. No evidences of gastrointestinal mucosal degeneration were found, however. The abdominal pain and fever was explained by the presence of extensive *Staphylococcic* abscesses in the liver, pancreas, and kidney. This bleeding tendency was not considered to be more extensive than is often seen in terminal leukemic states. It appears that prolonged doses of 6-mercaptopurine at a level of 3 to 4 mgm./kg. did not produce significant degenerative changes, and a dose of 12.5 mgm./

kg. in one 24 hour period did not seem to be injurious to the gastrointestinal tract. The areas of hepatic, renal, and pancreatic necrosis and abscess formation are not explained.

### *Summary*

(1) Eleven children, ages two to six, having acute stem-cell leukemia, have received 6-MP as part of their treatment.

(2) Seven children lost all evidences of their leukemia temporarily. All of this group either had previously become resistant to Aminopterin, or were receiving it in addition to the 6-MP.

(3) Two patients developed a partial remission: the one had received 6-MP alone, the other received cortisone for the first 13 days of the 6-MP therapy.

(4) Four patients previously resistant to 6-MP, Aminopterin, and cortisone were given 3 to 4 mgm./kg. of 6-MP without effect.

(5) The toxicity of 6-MP, apart from its effect on hematopoiesis, was limited to occasional episodes of mild buccal mucosal ulceration.

(6) An accidental dose of 250 mgm. (12.5 mgm. kg.) did not produce demonstrable gastrointestinal lesions.

### *Addendum*

Two of the three patients who were treated initially with both Aminopterin and 6-MP are still in complete remissions lasting seven months.

The third child thus treated remained in remission for approximately five months and relapsed. He was then given full doses of Aminopterin and was developing a leukopenia when he became febrile, had severe abdominal pain, and expired. At autopsy, he showed no convincing evidences of leukemic infiltration of his bone marrow or viscera, but showed an extensive thrombosis of his inferior vena cava with extension into both kidneys.

# CLINICAL EVALUATION OF 6-MERCAPTOPURINE

By Arthur Sawitsky and Charles R. Ream

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## *Introduction*

The adenine analog, 6-mercaptopurine (6-MP), has been shown to be an inhibitor of growth in various microbiological systems,<sup>1</sup> an *in vivo* inhibitor of growth of mouse sarcoma 180,<sup>2</sup> and an inhibitor of leukemic cell growth of several types of acute lymphocytic leukemia of mice.<sup>3</sup> Burchenal and his colleagues<sup>4</sup> reported on clinical studies of the antagonist and found it to be ineffective in chronic lymphatic leukemia, but to provide temporary remission, either partial or complete, in a relatively high percentage of patients having acute leukemia and chronic myeloid leukemia.

## *Methods of Study*

We have studied the clinical effects of 6-mercaptopurine in 14 children and 12 adults having acute or chronic leukemia. The drug was administered as a 50 mgm. scored tablet. The initial starting dose was 2.5 mgm./kg./day, calculated to the closest 25 mgm. amount.

In our first studies, the daily dose was divided morning and evening, but with later patients the entire daily dose was given at one time and was found to be of equal efficacy and toxicity. Patients in the hospital had daily hemoglobin and white-cell counts, while patients treated as outpatients had hemoglobin and leukocyte counts at least twice weekly or more frequently, as deemed necessary. Pretreatment bone-marrow examinations were made and followed with bone-marrow aspirations at about monthly intervals.

The criteria employed in this study were based upon the classifications recommended by this conference:

(1) Good hematologic and clinical remission. This state is defined as one in which the peripheral blood returns to normal and the marrow has a combined total of less than 30 per cent lymphocytes and stem cells.

(2) Partial remission in which clinical improvement is noted, together with some improvement of peripheral blood and marrow.

(3) Clinical remission only.

(4) Failure.

## *Results*

(1) In 14 children, a diagnosis of acute leukemia was made. Acute monocytic leukemia was diagnosed in one child 14 years of age. This patient was initially treated with 2.5 mgm./kg. but with no apparent response. After 10 days the dose was increased to 5.0 mgm./kg. and cortisone 200 mgm./day was added. There was no hematologic or clinical effect noted, and the patient died after 20 days of therapy. In the remaining 13 children, the ages varied from 18 months to 13 years. There were eight male and five female children.

Of this group, four children (28.5 per cent) have had six good hematologic and clinical remissions lasting 30 to 150 days.

Each of two patients of this group have had two remissions to the drug. One patient had previously become resistant to A-Methopterin and subsequently was treated simultaneously with both 6-mercaptopurine and cortisone. One patient had previously had a partial hematologic remission on cortisone therapy. One child has since expired, while the others presently are in either good or partial remission. Three of these four children remained in remission, even after cessation of drug administration for periods of 7 to 21 days.

A second group of five children have manifested six partial remissions of 18 to 100 days. Three patients of this group have had previous partial remissions in response to A-Methopterin and cortisone therapy. Cortisone was again added to the regimen of one of these children. Three of this group are dead, and two others are in continuing partial remission. Three children, two of whom had previously become A-Methopterin resistant, responded to 6-MP

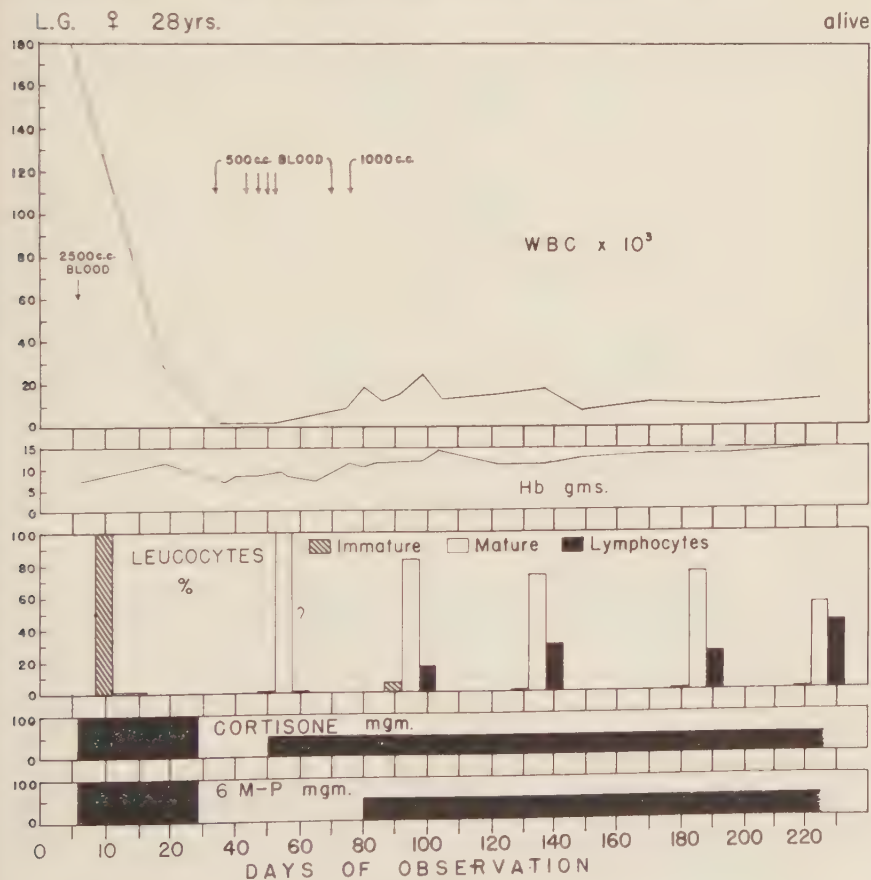


FIGURE 1



with clinical remission only, of 20 to 120 days. Toxicity in these 14 children was minimal, but noted to some extent in 57 per cent. Mild abdominal distress, *i.e.* mild diarrhea, abdominal pain, nausea, or gaseousness was noted in four patients. A burning sensation of the gums or tongue was noted in two, erythema of the gums in four, and irreversible bone-marrow aplasia in one. The patterns of response are similar to those which have been shown by other investigators contributing to this monograph.

(2) Six adults were diagnosed as having acute leukemia. One of these patients was a 26-year-old white female diagnosed acute monocytic leukemia. This patient received combination treatment because of severe mucosal bleeding, has had an excellent hematologic and clinical remission of 100 days, and is presently in remission. A period of severe bone-marrow aplasia preceded the onset of remission. Jaundice, with a clinical and a laboratory diagnosis of homologous serum hepatitis, further complicated the early treatment of this patient.

Case 1 (FIGURE 1) The patient, L. G., was a 26-year-old white female who was well until six months before admission. Some weakness and lassitude had been noted from that time. Four weeks before admission, exacerbation of weakness was observed and night sweats became troublesome. Two days before admission, severe gum and vaginal bleeding, as well as generalized

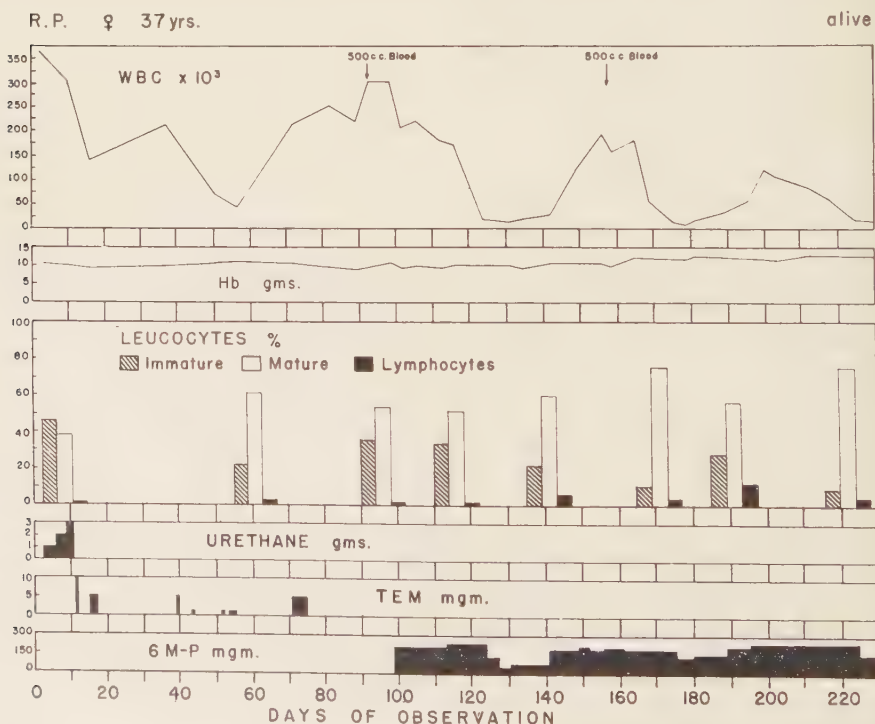


FIGURE 2

purpura and ecchymoses, were noted. On admission, lymphadenopathy was not marked. The gums were severely hypertrophic and bleeding. The spleen was not felt. The liver was palpable 2 cm. below the costal margin. The hemoglobin was 6.2 gm. per cent, and the white-cell count was 180,000, of which all were blast cells. Because of the severity of the bleeding diathesis, cortisone was added to the regimen of 6-MP, 2.5 mgm./kg. body weight. A blood transfusion was also given. The patient improved rapidly, and was discharged from the hospital on the 15th day. She failed to keep her O.P.D. appointment, since she felt well, and was next seen nine days later in the hospital for chills, fever, jaundice, and an infected index finger. The peripheral blood and bone marrow were found to be aplastic. A right lower lobe pneumonitis was also diagnosed. Cortisone was initially discontinued because of hallucinations, but was reinstituted 11 days later, when bleeding manifestations reoccurred. Antibiotics, blood, and the usual supportive measures for hepatitis were employed, and to our surprise the patient rallied and has since remained in good clinical and hematologic remission for the past 100 days.

Of the remaining five patients, there were four failures and one patient in whom a clinical remission only was obtained. This patient is still in remission after 160 days.

Case 11 (FIGURE 2). The patient, R. P., was a 37-year-old white female

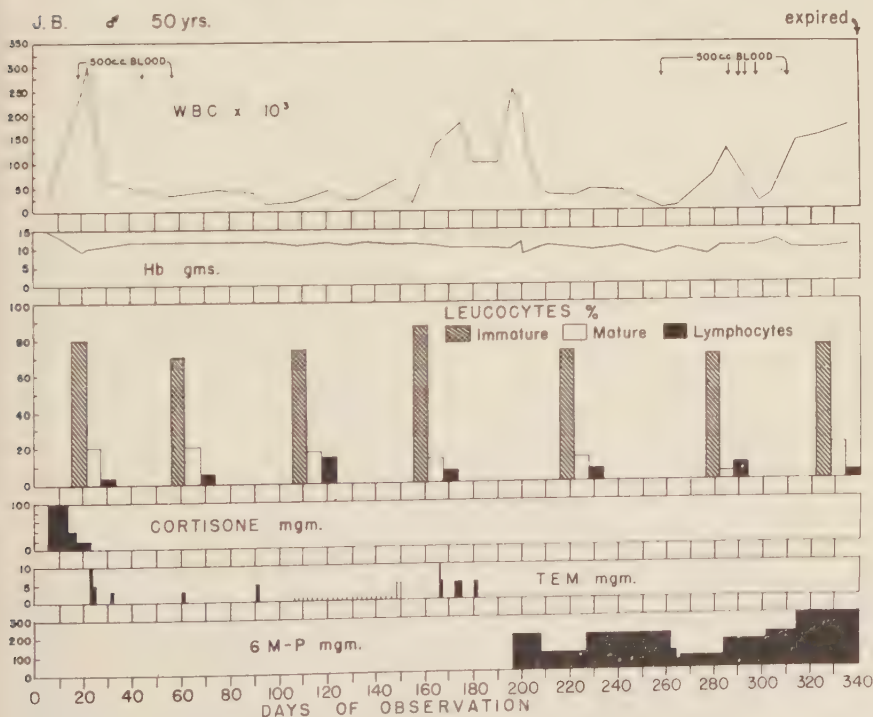


FIGURE 3

who had an acute onset of easy fatigability and progressive weakness of four weeks' duration. Fever and sweats have been noted for one week, while blurring of the vision of the right eye has been present for one day. Physical examination and laboratory findings corroborated a diagnosis of acute myelocytic leukemia. These values are shown on the accompanying slide. Urethane and tri-ethylene-melamine therapy led to transient hematologic benefit but to no clinical response. A change of therapy to 6-MP, however, produced rapid clinical improvement. Any reduction in daily dosage of 6-MP was followed almost immediately by rapid rise in peripheral white-cell count and loss of the patient's sense of well-being. This relationship between daily administration of the drug and clinical benefit was also noted with some others of the adult patients studied.

Three patients having chronic myeloid leukemia have had good clinical remissions, although the bone marrow and peripheral blood has remained typically leukemic. One of these patients had become completely refractory to urethane, cortisone, TEM, and X-ray therapy before trial with 6-MP. A clinical response was noted for 70 days and again, later, for 20 days, to a higher dosage before the patient became refractory to 6-MP and expired (FIGURE 3). Four other patients having terminal blastic leukemia showed transient clinical benefit of 10 to 20 days before resistance to increased dosage of 6-MP was noted and death occurred.

A 36-year-old white male having eosinophilic chronic myeloid leukemia had

	TYPE OF DISEASE	NUMBER OF PATIENTS	GOOD HEMATOLOGICAL & CLINICAL REMISSION	PARTIAL REMISSION	CLINICAL REMISSION ONLY	FAILURE		TOXICITY
						LESS THAN 3 WKS.	GREATER THAN 3 WKS.	
C H I L D R E N	ACUTE LEUKEMIA	13	4 (28.5%)	5 (35.5%)	3 (21.5%)	1 (7.1%)	0	8 (56.7%)
	ACUTE MONOCYTIC LEUKEMIA	1	0	0	0	1 (7.1%)	0	0
A D U L T S	ACUTE LEUKEMIA	5	0	0	1 (16.7%)	2 (33.3%)	2 (33.3%)	1 (16.7%)
	ACUTE MONOCYTIC LEUKEMIA	1	1 (16.7%)	0	0	0	0	0
	CHRONIC MYELOID LEUKEMIA	5	0	0	3 (50%)	0	2 (33.3%)	0
	CHRONIC MYELOID EOSINOPHILIC LEUKEMIA	1	0	1 (16.7%)	0	0	0	1 (16.7%)

FIGURE 4

previously obtained a clinical remission only, lasting 21 days, in response to ACTH and cortisone. Urethane then replaced the steroid therapy, but after three weeks the peripheral blood findings revealed increasing leukocytosis and immature cell eosinophilia. 6-MP therapy was accordingly instituted. A partial clinical and hematologic remission has ensued for the past 30 days.

### Discussion

The results achieved in this study, in which 6-mercaptopurine has been used alone, or in combination with cortisone either before or after other antileukemic agents have been used, are summarized in the accompanying chart (FIGURE 4).

From this chart it is apparent that 6-MP is another useful drug to be added to the antileukemic regimens now employed, which is capable of obtaining temporary benefit to some patients having acute and chronic leukemia who previously would have been unable to receive such temporary relief.

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## A CLINICAL STUDY OF 6-MERCAPTOPURINE

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The problem of medical care for patients afflicted with malignancies is in some respects different when handled by the clinician than when treated under the direction of a group doing investigative work. Prolonged periods of hospitalization for observation cannot usually be maintained when symptoms do not necessitate inpatient care. Therapy must be that which will produce relief of symptoms most rapidly. This problem is most prominent in cases of children having malignancies. The parents are young and have limited financial resources, and almost invariably they want the child treated as vigorously as possible. These factors were taken into consideration in all of the patients included in this report, and they explain why most of the children having acute leukemia received cortisone initially. It was not until some idea of the incidence of remission with 6-mercaptopurine was obtained, that this drug was used as the first therapeutic agent.

### *Results*

A total of 49 patients having several types of neoplastic diseases (TABLE 1) has been treated with 6-mercaptopurine. Forty-two of them were treated for longer than three weeks and were considered to have had an adequate therapeutic trial.

*Acute leukemia in children.* Twenty-four children having acute leukemia were treated with 6-mercaptopurine, and all except one were treated for longer than three weeks (TABLE 2). Only three of these children had not had previous treatment with cortisone and/or antifolic drugs. Their responses were classified as follows: (1) good remission: less than 10 per cent stem cells in the marrow and absence of symptoms; (2) partial remission: from 10 to 75 per cent stem cells in the marrow and improvement in the peripheral blood picture and symptoms; (3) no response: no appreciable hematologic or clinical response attributable to the drug. Improvement attributable to correction of anemia by transfusion or to control of infection was discounted as far as possible.

All except 3 of the 21 children treated with cortisone and/or antifolic drugs (usually Aminopterin) showed some response. Only one child in whom these were ineffective responded to 6-mercaptopurine, and this response was a partial one of three weeks' duration. Ten of the 24 children treated with 6-mercaptopurine developed good clinical and hematologic remissions which averaged 11 weeks hematologically and 12 weeks clinically. The duration of remissions ranged from 4 to 20 weeks, with a single exception (FIGURE 1). The exception was a male of eight and one-half years who had an initial remission of 35 weeks as the result of treatment with cortisone, followed by maintenance Aminopterin. His response to 6-mercaptopurine (1.6 mgm./kg.) was just as dramatic as to the original therapy. Hematologic remission lasted 28 weeks, and clinical remission, 30 weeks. During this prolonged period of therapy, no toxic mani-

TABLE 1  
PATIENTS TREATED WITH 6-MP

Diagnosis	Total	No. treated longer than 3 wks.
Acute leukemia		
Children.....	24	23
Adults.....	13	10
Chronic myelocytic leukemia.....	6	4
Chronic lymphocytic leukemia.....	1	0
Hodgkin's disease.....	4	4
Melanosarcoma.....	1	1
Total	49	42

TABLE 2  
ACUTE LEUKEMIA (CHILDREN)

Case No.	Response to previous treatment		6-MP therapy			Comments and course after 6-MP
	Steroid	Antifolic	Total dose (mg.)	Duration remission (wks.) and response		
				Clinical	Hematologic	
1	+	+	9,400	20 +	18 +	Clin. rem. antifol. plus cortisone
2	±	±	5,425	11 +	10 +	Lived 10 wks after 6 MP cortisone plus antifol.
3	0	0	250	0	0	Pneumonia
4	+	+	7,625	14 ±	8 ±	Needed cortisone for pain—died on 6 MP
5	+	±	4,725	7 ±	6 ±	Cortisone plus antifol—no response
6	+	+	4,250	9 +	9 +	Cortisone plus antifol—no response
7	+	±	5,600	13 ±	10 ±	Best response to HN <sub>2</sub>
8	+	+	5,250	13 ±	14 ±	Needed cortisone with 6-MP
9	0	0	4,750	7 +	3½ ±	Septicemia
10	+	+	4,480	12 +	14 +	Response again to cortisone plus antifol
11	+	0	8,350	15 +	15 +	Still in remission
12	No Rx	No Rx	2,800	7 +	6 +	Still in remission
13	No Rx	No Rx	3,150	8 +	7 +	Still in remission
14	No Rx	No Rx	3,650	4 +	6 +	Still in remission—needed cortisone for pain in legs (3 wks.)
15	+	+	10,500	20 +	18 +	Cortisone plus antifol—no effect=dose 6-MP—no response
16	+	+	3,875	8 ±	8 ±	Cortisone plus antifol.—partial plus TEM—no response
17	+	+	9,850	30 +	28 +	Lived 20 months. Cortisone plus antifol.—no response
18	+	±	3,925	14 +	12 ±	6-MP plus antifol.—12 wks clinical remission
19	+	No Rx	4,200	8 ±	7 ±	Antifol.—no response
20	±	No Rx	5,650	12 ±	14 ±	Antifol.—partial response
21	0	±	6,225	0	0	TEM—no response
22	±	+	2,870	0	0	Antifol. plus cortisone—no response
23	0	0	4,150	0	0	Cortisone—no response
24	±	+	7,640	0	0	Cortisone—partial response

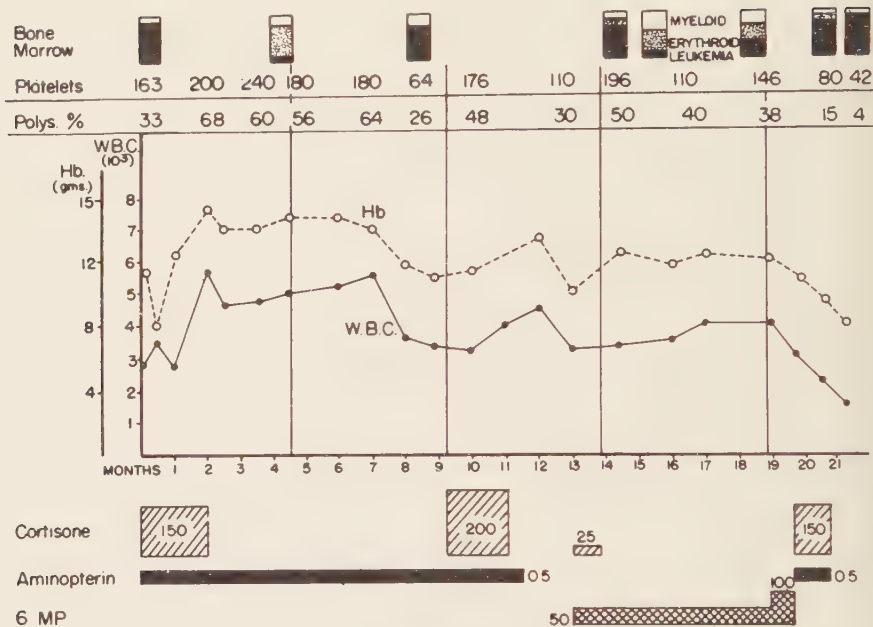


FIGURE 1

festations appeared. When relapse began, the dosage of 6-mercaptopurine was doubled (3.2 mgm./kg.). This treatment produced a leukopenia (WBC 1100) and some decrease in marrow cellularity, but no suggestion of remission. The three patients who did not have therapy before 6-mercaptopurine was started, developed good remissions ranging from four to eight weeks in duration on a dose of about 2 mgm./kg. One of these patients was in hematologic remission for two weeks before his bone pain disappeared.

All patients were given 2.0 mgm./kg. or less initially. The four patients who did not respond to 6-mercaptopurine and five of the others in whom the marrow response was only partial received 4.0 mgm./kg. without producing or increasing the degree of remission. No increase in the degree of response of the marrow and peripheral blood occurred after eight weeks of therapy, and in 9 of the 10 patients who developed good marrow remissions the response was complete in seven weeks. In general, the more complete the remission, the sooner hematologic improvement was evident. Comparison of the responses of the children who received an adequate course of 6-mercaptopurine with their response to cortisone and antifolics is shown in TABLE 3.

TABLE 3  
ACUTE LEUKEMIA (CHILDREN)

Therapy	Numberpatients	Good remission	Partial remission	No response
Cortisone.....	21	13 (61.9%)	4 (19.0%)	4 (19.0%)
Antifol.....	19	10 (52.6%)	5 (26.3%)	4 (21.0%)
6-MP.....	23	10 (43.5%)	9 (39.1%)	4 (17.4%)

Toxic manifestations were of two types:

(1) Eight patients developed white counts of less than 1800/cubic mm. which necessitated cessation of therapy from 4 to 12 days. The leukopenia was not accompanied by any appreciable decrease in platelet count.

(2) Four children developed mild vomiting and/or diarrhea which disappeared after three or four days without the drug.

No stomatitis or gastrointestinal bleeding was encountered. Skin lesions did not occur, and icterus did not appear in this group. Megaloblastic marrow changes were not found.

*Acute leukemia in adults.* Ten adults having acute leukemia were treated with 6-mercaptopurine for longer than three weeks (TABLE 4). Seven had acute lymphoblastic or myeloblastic leukemia. One patient (acute myeloblastic) had a partial remission which was predominantly clinical and indicated by increase in strength, decrease in abdominal discomfort and bone pain, and disappearance of fever. The hematologic portion of this response consisted of a drop in WBC from 45,000 to 14,100, a decrease in blasts from 45 to 20 per cent, stabilization of Hb and RBC, and rise in platelets from 20,000 to 76,000. The marrow response was less marked but the blasts decreased from 75 per cent to 60 per cent. The dose of 6-mercaptopurine was 2 mgm./kg. Two patients had clinical remissions without any hematologic change except decrease in white count.

One of the three patients having acute monocytic leukemia had a partial remission of six weeks' duration and the other two had some clinical improvement for six and eight weeks.

TABLE 4  
ACUTE LEUKEMIA (ADULTS)

	Good remission	Partial remission	Clinical remission	Failure	
				<3 wks. therapy	>3 wks. therapy
Acute lymphoblastic and myeloblastic	0	1 (6 wks.)	2 (3 & 10 wks.)	3	4
Acute monocytic	0	1 (6 wks.)	2 (6 & 8 wks.)		

TABLE 5  
CHRONIC LEUKEMIA (ADULTS)

	Good remission	Partial remission	Clinical remission	Failure	
				<3 wks. therapy	>3 wks. therapy
Myelocytic (a) early	0	2 (5 & 9 wks.)	0	1	1
(b) late acute	0	0	0	1	1
Lymphocytic	0	0	0	1	0



Two patients in this group developed diarrhea which, in one, was accompanied by vomiting. Both responded to interruption of therapy for four or five days. Two developed rather severe icterus, which was not satisfactorily explained, and both died without any decrease in jaundice. The liver sections at autopsy did not explain the mechanism of icterus.

*Chronic myelocytic and lymphocytic leukemia.* Four adults having chronic leukemia were treated with 6-mercaptopurine for longer than three weeks (TABLE 5). Two who had chronic myelocytic leukemia experienced partial remissions of five and nine weeks. The one patient having chronic lymphocytic leukemia died after 12 days of 6-mercaptopurine therapy.

*Miscellaneous malignancies.* Four patients having advanced Hodgkin's disease were treated with 6-mercaptopurine. The dosage varied from 1.5 to 3.4 mgm./kg. and the duration of therapy from 10 to 18 weeks. Three showed only leukopenia and further depression of their hypocellular marrows. A 12-year-old child who no longer responded to the usual measures became afebrile, active, and had a definite improvement in appetite for 12 weeks but no decrease in size of nodes.

One patient afflicted with melanosarcoma had a measurable disease in the size of his skin nodules for two months on a dosage of 0.9 mgm./kg

#### *Summary and Conclusions*

(1) 6-Mercaptopurine produced clinical and hematologic improvement in 23 children having acute leukemia who were treated for longer than three weeks. The remissions were good in ten of these patients.

(2) A response to 6-mercaptopurine was obtained in only one child who had been resistant to cortisone and folic acid antagonist.

(3) When relapse occurred after 6-mercaptopurine induced remission, increased dosage did not prolong the remission.

(4) No good remissions were obtained in 10 adults having acute leukemia who were treated for longer than three weeks. Two had partial remissions and four had short clinical remissions.

(5) Two of four adults having chronic leukemia treated for longer than three weeks had partial remissions.

(6) Four patients having Hodgkin's disease were not influenced by 6-mercaptopurine.

(7) The toxic manifestations were leukopenia, gastrointestinal disturbances, and possibly icterus.

(8) 6-MP alone is not the therapy of choice to be used initially in any type of leukemia when symptomatic relief, with the purpose of getting the patient quickly on an outpatient basis, is attempted.

# THE TREATMENT OF LEUKEMIA WITH 6-MERCAPTOPURINE\*

By Quin B. DeMarsh

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Nineteen cases of various types of leukemia have been treated to date with 6-mercaptopurine (6-MP). With some exceptions, the patients had been treated with A-Methopterin, steroids, or radiation in some form, previous to treatment with 6-MP. In chronic myelogenous leukemia, the drug was used only in those cases which were resistant to other forms of therapy and were entering the blastic stage. The majority of the cases classified as acute myelogenous leukemia of adults was treated initially with 6-MP. All of the cases of monocytic leukemia were treated primarily with this drug. The children were all classified as acute lymphatic leukemia, and all had been treated for periods of one year or more with steroids and antifolics.

The patients were for the most part private patients treated as outpatients, and hospitalized only when acutely ill or terminal. Complete peripheral blood studies and bone marrows were done initially in all cases. Marrow aspirations were done repeatedly in some cases, especially in those in which the hemotologic response, as evidenced by changes in the peripheral blood, was most striking. However, these tests were not repeated in children. It was rare to find a patient who responded little or not at all to the drug but, by and large, remissions of long duration were relatively rare. The most dramatic effects were noted in some acute leukemias of adults and of childhood. The results in four cases, classified as acute or subacute monocytic leukemia, were gratifying in that previous methods of treatment for this disease have invariably produced a lesser effect.

In the following case reports, the figures for the leukocyte, erythrocyte, and platelet counts are the number cu. mm. of blood. The hemoglobin reported is the gm./100 ml. of blood, and the dosage of 6-MP is mgm./day.

## *Chronic Myelogenous Leukemia*

N. B., a female aged 66, whose disease was first discovered in January 1952, was controlled with radioactive phosphorus until she entered her resistant phase. 6-MP therapy was begun with 125 mgm. on May 15, 1953. A total of 1,700 mgm. was administered during a 15 days' period. At the onset of 6-MP therapy her hemoglobin level was 10 gm. and the leukocyte count was 316,000. During the period of therapy the number of leukocytes rose to a maximum of 700,000, and at the end of the treatment period it was 400,000. Treatment was discontinued at the request of the patient. The count continued to fall till it reached a level of 30,000. There was no regeneration of hemoglobin or of platelets during this period, and the patient did not improve

\* The author wishes to make acknowledgment to Doctors Clement A. Finch and Daniel Coleman, Department of Medicine, University of Washington School of Medicine for reporting the data of five cases treated by them. Acknowledgment is also made to the Burroughs Wellcome Company for supplying the 6-mercaptopurine used in these cases.

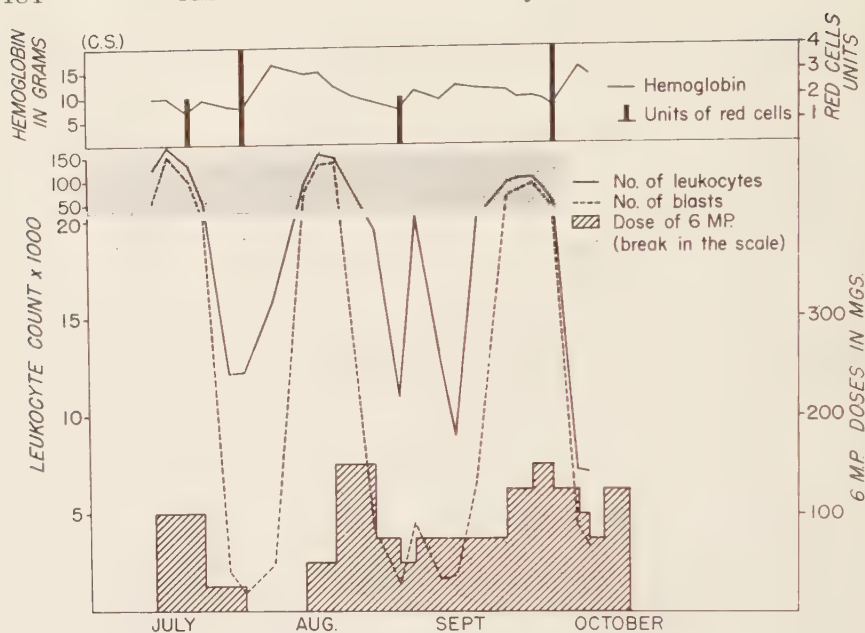


FIGURE 1

clinically. The leukocyte count gradually rose to 600,000 before the patient expired.

C. S., a 41-year-old female, was diagnosed as chronic myelogenous leukemia in January 1951, at which time her leukocyte count was 15,000. She was treated initially in October 1951 with radioactive phosphorus. She responded well and was kept in remission till the latter part of June 1953 with administrations of  $P^{32}$  whenever this was indicated. 6-MP was started on July 13, 1953, with 100 mgm. doses daily. Within ten days her leukocyte count had fallen from 134,000 to 49,000, and the dosage was decreased to 25 mgm. This type of program was maintained for a period of approximately two and one half months. This patient invariably responded favorably both clinically and hematologically, although transfusions were required at intervals to maintain her hemoglobin. Her hematologic course is recorded graphically in FIGURE 1. She eventually developed refractoriness to the medication and died four and one half months after the onset of 6-MP therapy. She had received a total of 7,100 mgm. in 79 days of therapy.

E. L., a 67-year-old female, had received intermittent X-ray treatment for one and one half years. She entered the hospital having a splenic infarct and a blastic crisis on Sept. 2, 1953. She was given X ray to the spleen, combined with 100 mgm. of 6-MP for five days. The leukocyte count fell from 145,000 to 30,000 ten days after onset of therapy, with a rapid rise to 190,000 four days thereafter. At this time 6-MP alone was started at 50 mgm. The leukocyte count continued to rise gradually, so the dosage was raised, four days later, to 100 mgm. and, four days later, to 150 mgm. The count climbed

to 340,000 and, after nine days, the dose was increased to 200 mgm. This second course lasted two weeks, and there was an increase in platelets during this time. The patient felt somewhat better for a short period, but then developed nausea and vomiting. She stopped her medication because of these developments. The leukocyte count rose rapidly to 735,000. The patient expired 60 days after the first administration of the drug, after receiving 3,850 mgm. during 29 days of therapy.

### *Acute Myelogenous Leukemia*

Six of the cases treated were classified as acute leukemia of adults, probably acute myelogenous leukemia.

M. W., a 60-year-old white female, was first seen in the hospital on Aug. 6, 1953. She gave a history of repeated upper respiratory infections treated by antibiotics at home and finally hospitalized because of pneumonia of the upper left lobe. A leukocyte count was 1,000 with 14 per cent blasts, hemoglobin was 11 gm., platelets were slightly decreased, but bleeding was not a problem clinically. On Oct. 15, 1953, the patient was started on 100 mgm. of 6-MP a day for a period of 17 days. A marrow aspiration done on the first day of therapy consisted of nothing but blast cells considered to be myeloblasts. The leukocyte count rose in two days to 3,250, then fell slowly during the next four days to 1,200, at which time the hemoglobin was 7.8 gm. The patient was given four units of red cells which raised the hemoglobin to 13.6 gm. The drug was discontinued. The patient's leukocyte count continued to fall during the next ten days to a minimum of 700. The hemoglobin again was a low of 7.8 gm., and she was given another four units of red cells. The leukocytes gradually increased over the next two weeks to 2,000, with a normal differential. One week later, on September 22, the hemoglobin was 15.6 gm., leukocyte count was 9,900 with a normal differential, and there were a normal number of platelets. Except for a slight increase in plasma cells, the bone marrow aspiration done at this time was considered normal. The patient felt completely well and had gained 22 lbs. in weight. Blood counts were done at weekly intervals with normal results. The diagnosis was changed to a probable leukemoid reaction, since the patient did so well. However, three weeks later, on Oct. 15, 1953, the leukocyte count had risen to 15,000 with 2 per cent blasts. The patient was given a 10-days' course of 100 mgm. of 6-MP daily. Another remission occurred, which lasted till Nov. 17, 1953, at which time a few blasts were seen in the peripheral blood. Another 700 mgm. of 6-MP was administered during the next week. The leukocyte count continued to remain normal, and a marrow aspiration done on Dec. 16, 1953, was again considered normal. The patient subsequently had a relapse and was given the fourth course of 6-MP starting on Jan. 28, 1954, for 11 days at 75 mgm. For 22 days after this course, she was controlled with 50 mgm. daily. A marrow aspiration on March 30, 1954, was considered normal after four weeks without therapy. This patient has had the best result with 6-MP to date. She is apparently very sensitive to small doses of this drug. To date she has received a total of 5650 mgm. in 70 days. Her hematologic course is depicted in FIGURE 2.



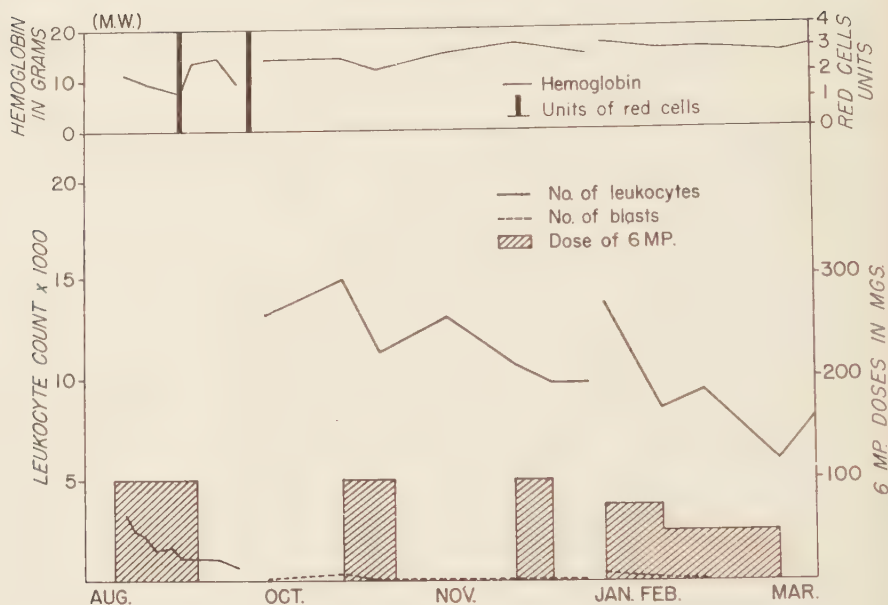


FIGURE 2

L. E., a 35-year-old white female, was first seen on Dec. 8, 1953. She gave a history of undiagnosed, macrocytic anemia of about six weeks' duration. She had been treated previously with 100 gammas of B12, given intramuscularly, without response. Physical examination was negative except for pallor. The hemoglobin was 7 gm., erythrocyte count was 1,800,000, hematocrit 20 per cent, leukocyte count was 3,500 with 10 per cent blasts, and a marrow aspiration revealed 90 per cent blast cells, considered to be myeloblasts. The platelet count was slightly decreased. The patient was transfused with six units of red cells and placed on 150 mgm. of 6-MP. She was treated as an outpatient until a left gluteal abscess developed, probably from the previous injection of B12. She was hospitalized a week after first having been seen, treated with antibiotics, and the abscess was drained surgically. She seemed to improve and was allowed to go home for Christmas. When seen on Dec. 31, 1953, the leukocyte count was 1,100 with 2 per cent blasts, hemoglobin was 12.2 gm., and she had a fever of  $102^{\circ}$  F. The gluteal abscess had become worse in spite of continuous antibiotic therapy. She was hospitalized, 6-MP was discontinued, and the number of leukocytes fell rapidly to 300. Her gluteal abscess did not respond to further treatment and the patient died on the fourth day after hospitalization, 25 days after treatment was first begun and after 15 days of 6-MP therapy for a total of 1,650 mgm. Autopsy revealed hydrothorax, focal infarction of the upper and middle lobes, pulmonary edema, and a gluteal abscess. The bone marrow was aplastic. It was felt that this was a possible aplasia of the marrow attributable to 6-MP.

D. A., a 15-year-old white male, had massive adenopathy when first seen, a

leukocyte count of 250,000 and 9 gm. of hemoglobin. He had a large abscess in the right axilla stemming from a furuncle on the right arm. A pure culture of *Staphylococcus albus* was grown from the abscess. He was treated with antibiotics, given A-Methopterin for a period of five days, and then switched to 6-MP, initially at 150 mgm., but in varying doses as symptoms improved. This first course lasted for 44 days, with a total of 4,525 mgm. The leukocyte count on changing drugs was 99,250 with 86 per cent blasts. The patient was kept on a maintenance dose of 50 mgm. for the last six days of this first course. At the end of the 44th day he had neither adenopathy nor splenomegaly. During this period he required intermittent transfusions to maintain his hemoglobin. In the meantime the platelet count had risen to a satisfactory level. The drug was discontinued, and the patient continued in fair remission for 41 days, when there was an occasional blast in the peripheral blood and a slight decrease in platelets. The patient was put on 150 mgm. of 6-MP for seven days. The clinical and hematological remission was excellent, the platelet count and hemoglobin returning to normal. On March 30, 1954, seven days after the termination of the second course, a bone-marrow aspiration revealed marked leukemic involvement, although the hemoglobin and platelet count were normal, and there were no blasts seen in the peripheral blood smear.

A. G., a 56-year-old white female, was initially given 400 mgm. of cortisone daily for 18 days, and then 100 mgm. daily for a period of 30 days. Her purpura disappeared, the leukocytes dropped in number from a high of 70,000 to 5,600 during this period of cortisone therapy. There was considerable subjective improvement. The patient was then switched to 6-MP beginning with 150 mgm. for 55 days, for a total of 8,250 mgm. During this period there was objective improvement. The platelet count rose, and the hemoglobin maintained itself. After the 76th day, the hematocrit was 28 per cent, the leukocyte count was 2,670, and the platelet count was 376,000. Within another week, however, the hematocrit was falling, the leukocyte count was 600 and, by the end of the third week, the platelet count was 40,000 and the leukocyte count was 154. This patient expired on April 6, 1954, 125 days after the onset of 6-MP therapy.

K. M., a 32-year-old Japanese male, was treated with 150 mgm. of 6-MP for eight days, for a total dosage of 1200 mgm. His admission hemoglobin was 12 gm., leukocyte count was 150,000, with 95 per cent blasts. In spite of continued therapy, the number of leukocytes continued to rise till it was 450,000 on the day of death. Platelets were absent. No clinical or hematologic effect was noted from the administration of the drug.

G. S., a white male aged 76, was treated, for a period of about 15 days, with total body irradiation with little effect upon the leukocyte count, platelet count, or hemoglobin level. Transfusions were necessary. He was given 6-MP at 100 mgm. for 48 days for a total of 4,800 mgm. After 14 days of treatment, his leukocyte count fell gradually from a high of 135,000 to a low of 15,000 on the 45th day. As a complication of his disease, he had acute hemolytic anemia with a red-cell life span of 15 days. Toward the end of his disease, he was given 225 mgm. of cortisone daily, which did not control the

hemolytic process. He had no clinical improvement on either drug, acquired bronchial pneumonia, and died 48 days after the onset of therapy.

### *Monocytic Leukemia*

Patient C. M., a 56-year-old white male, was treated for a period of only five days with 175 mgm. of 6-MP daily. At the onset of therapy the leukocyte count was 30,000 with 78 per cent monoblasts, 6 per cent neutrophils, and a platelet count of 17,000. At the end of treatment the number of leukocytes had decreased to 12,350 with 69 per cent monoblasts, 14 per cent neutrophils, and a 12,150 platelet count. The leukocytes continued to decrease in number, as did the hemoglobin, in spite of transfusions. The platelet count, however, rose to a high of 158,000. Two weeks after cessation of therapy with 6-MP, the leukocyte count was 2,200 with 17 per cent monoblasts, 7 per cent neutrophils, and 76 per cent lymphocytes. On Feb. 29, 1954, 56 days after the end of the first course with 6-MP, the leukocyte count was again elevated to 18,000 with a high percentage of monocytes. The platelet count was falling, and the patient did not feel as well as he had. He was given a second course of 175 mgm. of 6-MP daily for five days. The leukocyte count fell rapidly to 2,500, and the platelet count again returned to near normal. There was clinical improvement. The patient was not under our direct control during this second course. He apparently had had dramatic effects on small doses of 6-MP.

Patient J. P., a 57-year-old white male, was seen after several months of illness, during which he had no medical attention. The diagnosis of leukemia had been made on the first visit to his family physician. The patient received 6-MP, more or less continuously, from Oct. 27, 1953, to Feb. 6, 1954, together with transfusions. The platelet count was never a problem, remaining more or less normal throughout his illness. His response to treatment was considered good. He received a total of 9,230 mgm. in 105 days. Without informing us, however, he stopped medication of his own accord. His disease fulminated, and he died on the 116th day after onset of 6-MP therapy in the hospital, where he had been placed four days prior to death. His hematologic course is depicted in FIGURE 3.

G. G., a 77-year-old white male, was first treated with 6-MP at 150 mgm. for three days. At the start of therapy, he had a leukocyte count of 51,800 with 80 per cent monoblasts, and the bone marrow was almost completely replaced by monoblasts. At the end of the three days, the number of leukocytes had dropped to 29,750. The platelets had decreased, as had the hemoglobin. Treatment with 6-MP was started four days later at 150 mgm. and maintained until five days before the patient died, on the 22nd day after onset of therapy. The leukocyte count remained steady at around 20,000 but, the day before death, it was recorded as 71,000, all of which were mononuclear cells. The patient's platelet count remained decreased, and bleeding was prominent. He had diarrhea, a focal involvement of the large bowels, and agranulocytosis. He had received a total of 1,950 mgm. in 13 days.

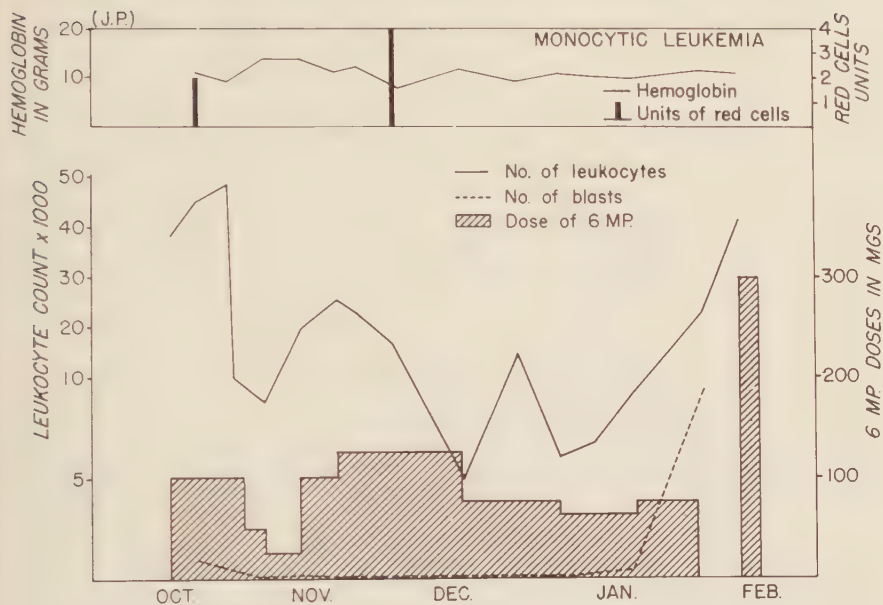


FIGURE 3

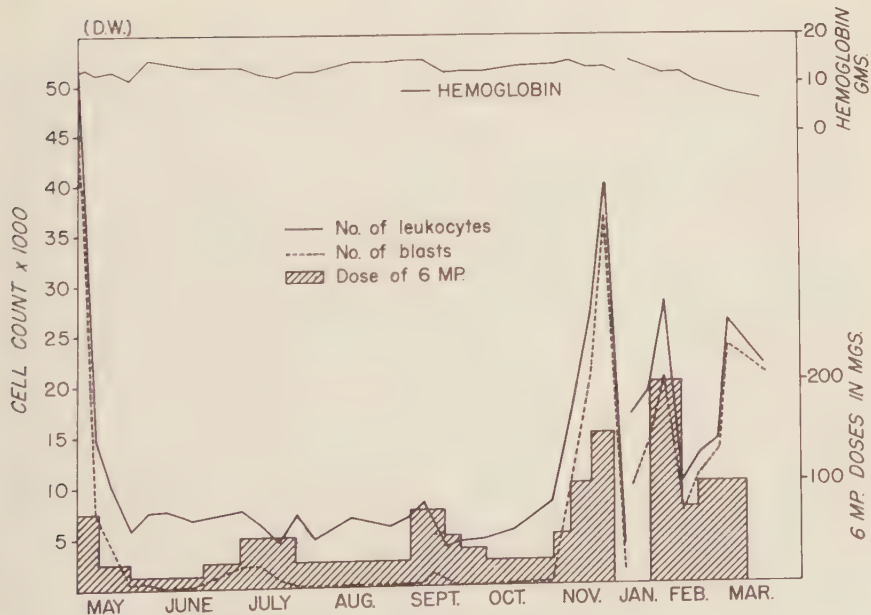


FIGURE 4



Patient J. D., a 49-year-old white male, had been ill for some time before he was first seen. On first examination, he had an extensive disease of the gums. The hemoglobin was 10.9 gm., leukocyte count was 8,900 with 49 per cent monocytes and monoblasts, and platelets were markedly decreased. The stool was positive for occult blood. The patient was given numerous transfusions because of the intestinal bleeding. He was placed on 100 mgm. of 6-MP and, later, on 150 mgm. a day for a total of 1550 mgm. in 13 days. There was no change in the number of leukocytes or platelets, and the patient continued to bleed. He also had acquired hemolytic anemia with a positive Coombe's test. He died 25 days after onset of therapy, of gastrointestinal hemorrhage.

### *Acute Lymphatic Leukemia*

Six patients were classified as having acute lymphatic leukemia. Two of them were adults and four were children. All except one case were treated for considerable periods with cortisone or A-Methopterin until they became refractory to both medications. The two adults had little or no response to the drug. All of the children received some benefit, in that their leukocyte counts were lowered and the percentage of blasts decreased somewhat, but the dosage level was difficult to regulate. The case treated the longest, with the best results and the longest intervals of remission, is depicted graphically in FIGURE 4.

### *Summary*

Nineteen patients having various types of leukemia were treated for varying periods of time with 6-mercaptopurine. The average dose was 2.5 mgm./kgm.

While some effect can be produced on the peripheral blood in terminal chronic myelogenous leukemia, the clinical remissions were not of long duration. Of the six cases of acute myelogenous leukemia treated, two were of such short duration that the effects of the drug could not be evaluated. One patient has been controlled very well for a period of several months on intermittent therapy, and one patient died possibly of toxic effects of the drug. One patient has had repeated clinical and hematologic remissions, and has been kept in excellent health by brief periods of treatment on small doses of this drug.

Of the four cases of monocytic leukemia treated, two had short treatment periods with little or no clinical effect. In one case the leukocyte count of the patient was controlled for a considerable length of time on maintenance therapy, but with little change in the bone marrow, and the fourth patient has been controlled with short treatment periods of small doses of the drug.

Six patients having acute lymphatic leukemia were treated, all of whom had been treated previously, over a long period of time, with other drugs. Two were adults and four were children. The two adults showed little or no beneficial effect from the drug and, of the four children, three had brief clinical

remissions, while one had a clinical remission on maintenance therapy for about three and one-half months.

The drug appears to be most effective in the acute leukemia of adults and in monocytic leukemia. Some patients appear to be much more sensitive than others to the effects of the drug, and these are the cases which have derived the most benefit. We have had only one case in which a lasting hematologic effect has occurred.

# THE EFFECT OF 6-MERCAPTOPURINE IN ACUTE LEUKEMIA OF CHILDREN AND ADULTS: A PRELIMINARY REPORT\*

By N. L. Petrakis, D. A. Wood, S. R. Mettier, W. C. Deamer, S. A. Gellert, J. G. Li, and M. E. Leonard

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The antipurine compound 6-mercaptopurine (6-MP),<sup>†</sup> synthesized and developed by Hitchings and his colleagues<sup>1-4</sup> has been found to inhibit the growth of a wide variety of animal tumors<sup>5, 6</sup>. Burchenal *et al*<sup>7</sup> reported 6-MP to be effective in inducing temporary remissions, complete and partial, in approximately 40 per cent of children having acute leukemia and in a few adults having acute leukemia and terminal granulocytic leukemia. Hall and his associates<sup>8</sup> reported an initial remission rate of 63.6 per cent with 6-MP in adults afflicted with acute leukemia.

The present preliminary report on the effect of 6-MP in children and adults having acute leukemia is based on studies made since October 1953, when sufficient amounts of this agent became available to us for clinical trials. The results obtained in this limited group of patients are similar to those reported by the authors previously mentioned.

## Materials and Methods

Fourteen patients were treated with 6-mercaptopurine. The diagnoses were based on the clinical history and physical, hematological, and bone-marrow examinations. Six children had acute lymphocytic leukemia and one had acute monocytic leukemia. Of the seven adults studied, two had acute lymphocytic leukemia, two had acute monocytic leukemia, two had granulocytic leukemia in acute exacerbation, and one had multiple myeloma. The sex, age, duration of disease, prior therapy, and general condition at the time of therapy with 6-MP are shown in TABLE 1. Most patients were observed in the hospital for a week prior to therapy and were followed in the outpatient clinic as soon as practicable after starting treatment on 6-MP. Initially, peripheral blood studies were made at least three times weekly and bone-marrow aspirations were made weekly. Later, blood studies were performed at least once a week, and bone marrows were examined every two to three weeks. The daily dose of 6-MP administered was from 2 to 4 mgm./kg., most patients receiving 2.5 to 3 mgm./kg./day. Treatment was initially given in two to three week courses of therapy. Most recently, continuous maintenance therapy has been instituted. Repeated courses were given in the event of physical signs of clinical relapse, developing or refractory anemia, and persistence of blasts in the bone marrow. Antibiotics and transfusions

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† The authors wish to thank Doctor Donald S. Searle, Burroughs Wellcome & Co. (USA), Inc. for generously providing the 6-mercaptopurine (Purinethol<sup>®</sup>) employed in these studies.

were employed when clinically indicated. In four patients, 6-MP therapy was supplemented by local X ray, GT-41 (Myleran<sup>®</sup>), and cortisone.

### Results

*Acute leukemia in children.* Seven children having acute leukemia received 6-mercaptopurine. Little toxicity was encountered except for mild transient diarrhea in three patients during the first week of therapy. A few small buccal ulcerations were observed in patient No. 6 (TABLE 1), who did not respond to therapy. Two of the children had received previous therapy in the form of radiophosphorus and triethylene melamine.

Two children (Nos. 1 and 2) had partial remissions under 6-MP, characterized by clinical improvement, such as disappearance of lymphadenopathy and splenomegaly, improvement of appetite, gain in weight, and increased physical activity. These patients had only partial improvement in hemoglobin levels, and they continue to have greater than 30 per cent blast cells in the bone marrow. Marrow cellularity, as evaluated from smears, appeared to be reduced after prolonged therapy with 6-MP. The duration of the partial remission was 15 and 7 weeks, respectively. The hematologic response to the initial course of therapy in patient No. 1 is shown in FIGURE 1. These two patients are now on daily maintenance therapy.

Three children (Nos. 3, 4, and 5) had clinical remissions only, without improvement in hematologic status. The duration of clinical improvement was 3, 7, and 10 weeks, respectively. In child No. 5 a clinical remission was subsequently produced with cortisone, with improvement in the hematologic status.

One child (No. 6) did not respond to 4 mgm./kg./day of 6-MP given for 19 days, except for an apparent decrease in cellularity of the marrow smears. Patient No. 7, having acute monocytic leukemia with spinal cord infiltration, paraplegia, and cord bladder, expired before adequate therapy could be given.

*Acute leukemia in adults.* Of the seven adults treated, three patients who were considered to have received adequate trials of therapy had clinical and hematologic improvement.

Patient No. 10, having acute monocytic leukemia, developed a complete and striking clinical and hematologic remission, including return of the bone marrow to normal (FIGURE 2). The duration of the initial remission was approximately 10 weeks before the occurrence of hematologic relapse, manifested by falling hemoglobin and platelet count, and clinical enlargement of the spleen. Subsequent therapy produced only transient clinical and no hematologic improvements. Cortisone was employed terminally without effect.

Patient No. 11, having acute monocytic leukemia, developed a clinical remission with some improvement of his hematologic status, consisting of a rise in platelet and decrease in leukocyte count, with only partial response of the bone marrow and no effect on the hemoglobin level.

Patient No. 8, having acute lymphocytic leukemia, showed a striking fall in leukocyte and platelet count, a decrease in splenomegaly, and improvement



TABLE 1  
6-MERCAPTOPURINE IN THE TREATMENT OF PATIENTS WITH LEUKEMIA

Case	Sex & Age	Prior treatment	General condition	Duration disease months	Dose Total dose schedule mg.	Dose mgm./kg.	Additive treatment	Initial clinical response					Spleen size		Hb. response	Platelet response	Duration of remission	Remarks
								Good hemat. clin. remission	Partial remission	Clinical remission only	Failure <3 wk. treatment	Failure >3 wk. treatment	Before cm.	After cm.				
Acute Lymphocytic Leukemia—Childhood																		
1 POW	F 2	0	Poor	2 4	14/14 700 19/19 950	4	Blood		X				4	1	+	+	15 weeks	Continues in partial remission. On maintenance.
2 POR	F 2	0	Fair	5 5 1 2	28/28 1400 28/28 1400	3	Blood		X				3	0	+	+	7 weeks	Continues in partial remission. On maintenance
3 BAR	F 5	TEM	Fair	9 11 13 14	12/12 655 10/10 500 24/24 1200	2.5	Blood terramycin			X			7	2	0	+	3 weeks	Expired one month after last therapy.
4 SHO	F 4	P <sub>22</sub>	Poor	3	24/24 1200 16/16 800	3.0	Blood penicillin			X			3	0	0	0	7 weeks	Expired 9 weeks after last therapy
5 BEN	M 4	0	Fair	2	30/30 1000	2.5	Blood penicillin cortisone			X			7	2	0	0	10 weeks	Had subsequent clinical and hematological response to cortisone.
6 LOG	F 2.5	0	Poor	2 3 4	13/13 2145 6/6 300 7/7 350	4.0	Blood antibiotics cortisone				X		1	1	0	0	None	Expired three weeks after last therapy.
Acute Monocytic Leukemia—Childhood																		
7 LAR	F 9	0	Poor	1 2	11/11 425 10/10 500	2.0	Blood local X ray				X		2	8	0	0	None	Inadequate trial. Expired while on therapy.

TABLE 1 (continued)  
Acute Lymphocytic Leukemia—Adult

8 AMA 23	F	0	Poor	2	11/11, 14/14	2200 3200	2.5	Blood	X	3 15	0 0	+	6 weeks	Continues on partial remission. On maintenance therapy. Inadequate trial. Expired while on therapy.
9 O'H 33	M	X-ray Medi- ast.	Poor	1.5	7/7	2800	2.5	Blood	X	0	0	0	None	
Acute Monocytic Leukemia—Adult														
10 ATC 29	M	0	Fair	3	18/18 13/13	2000 1950	2.5	Blood con- tinue anti- biot. Blood	X	8	0 0	+	10 weeks	Had complete remis- sion. Expired three weeks after last therapy. Clinical Continues in clinical remission. On maintenance therapy.
11 KIN 31	M	0	Good	8 1 2	17/17 11/11 14/14 28/28	2550 550 2800	3.5	Blood	X	0	0	0	3 weeks	
Chronic Granulocytic—Terminal—Adult														
12 BUR 46	F	GT-41	Poor	16	13/13 9/9	1650 680	2.0	Cortisone		X	9 0	0 0	0 0	Expired one month after last therapy Inadequate trial. Expired three days after start of ther- apy
13 BAC 33	F	pn	Poor	60	3/3	450	2.0	GT-41	X		0	0	0	
Multiple Myeloma														
14 STA 49	M	0	Poor	24 25	12/12 13/13	1450 450	2.5			X	0 0	0 0	0 None	No response to 6-MP. Currently on urethane.

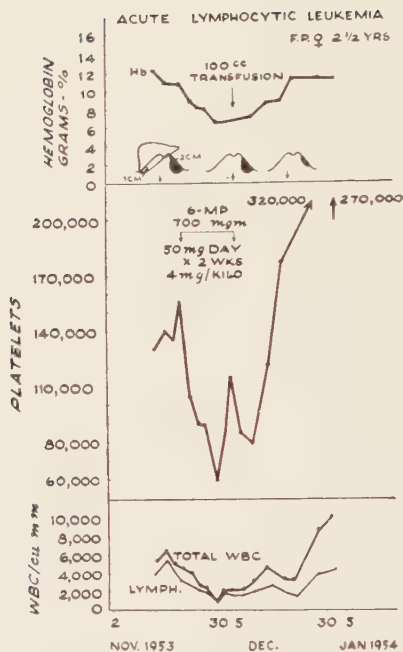


FIGURE 1. Case No. 2 Acute lymphocytic leukemia in a 2½ year old female showing hematologic remission in the peripheral blood and decrease in hepatosplenomegaly produced with 6-MP. The bone marrow did not show improvement.

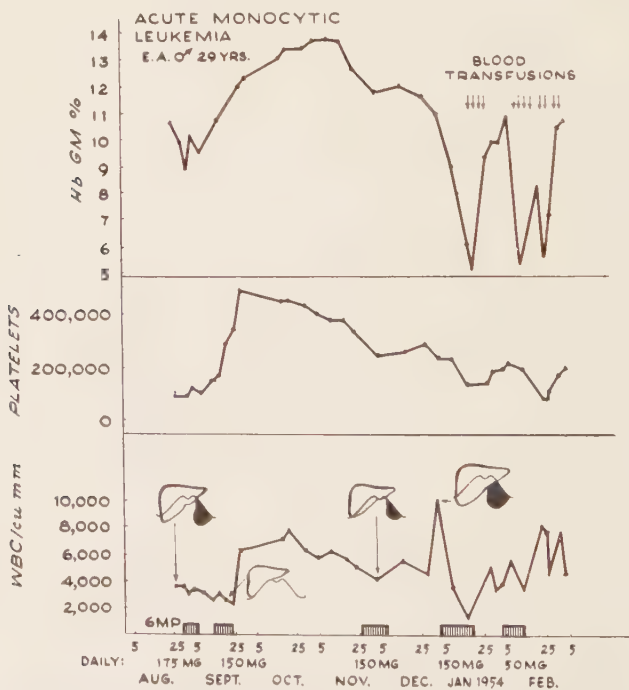


FIGURE 2. Case No. 10. Acute monocytic leukemia in a 29-year-old male who showed complete clinical and hematologic remission for ten weeks following 6-MP therapy.

in clinical status after two weeks of therapy with 2.5 mgm./kg. 6-MP. Hemoglobin response did not occur. The clinical remission persisted for three weeks before splenomegaly increased, and the anemia became more severe. The patient showed a prompt clinical and partial hematologic response to a second course of therapy. The bone marrow was not materially affected by either course of therapy.

Patient No. 12, having terminal granulocytic leukemia, showed a prompt reduction of the granulocyte count from 175,000 to 20,000 cubic mm. after 22 days of therapy with 2.0 mgm. kg. 6-MP, but failed to show clinical or other hematologic improvement, and expired a month following the last 6-MP therapy. Patient No. 14, having multiple myeloma, showed progression of bone lesions and anemia during 6-MP therapy given in two courses over a two-month period. A decrease in normal granulocytic elements of the bone marrow was observed during the first course of treatment.

Patients No. 9 and 13 expired before adequate trial with 6-MP could be given, but each patient showed a striking drop in leukocyte count within three to five days after starting treatment.

### *Discussion*

The present limited summary of our experience with 6-mercaptopurine in the treatment of acute leukemia of children and adults is in agreement with earlier published reports cited above. 6-MP produced partial clinical remissions and hematologic improvement, or clinical improvement only, in five of seven children afflicted with acute leukemia, in two adults having acute monocytic leukemia, and in one adult afflicted with acute lymphocytic leukemia. No clinically significant effect was observed in one patient having multiple myeloma, in two patients having terminal granulocytic leukemia, and in one patient having acute lymphocytic leukemia. The duration of remissions induced was from three to fifteen weeks. These patients experienced decreased irritability, improvement in appetite, and a feeling of well-being. There was an associated decrease in the clinical findings of fever, lymphadenopathy, splenomegaly, and signs of bleeding.

The dose levels of 6-MP employed were from 2 to 4 mgm./kg./day. Most patients received two- to three-week courses of therapy, but more recently have been maintained on more or less continuous therapy. Leukocyte depression occurred during the first one-week to three-weeks of treatment in all patients, but treatment was continued despite the leucopenia. Bone-marrow hypocellularity appeared in most patients who were maintained on prolonged therapy, which was reversible on discontinuing the drug.

Little evidence of toxicity attributable to 6-MP was encountered, other than mild diarrhea in three patients and stomatitis in one patient. Combined therapy of 6-MP with other antileukemic agents was not employed. Cortisone was given to two patients after lack of further response to 6-MP therapy.

Based on the present limited experience with 6-mercaptopurine, this agent was found to show definite, objective effects upon the course of the leukemic process, which are of sufficient interest for the continuation and expansion of investigation.



*Conclusions*

(1) 6-Mercaptopurine in doses of 2 to 4 mgm./kg./day was employed in the treatment of seven children having acute leukemia, and in seven adults having acute leukemia, terminal granulocytic leukemia, and multiple myeloma.

(2) Partial remissions were induced in five children, which persisted for 3 to 15 weeks. Two adults having acute monocytic leukemia and one having acute lymphocytic leukemia experienced clinical and hematologic improvement of 3 to 12 weeks' duration.

(3) The toxicity to this agent at the dose levels employed was limited to transient diarrhea in three patients, and mild stomatitis in one patient.

*Acknowledgment*

The authors wish to express their appreciation to Alice McBride and to Lois J. Folstad for their technical assistance in this study.

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# THE CLINICAL AND HEMATOLOGIC RESPONSE OF PREVIOUSLY TREATED LEUKEMIC SUBJECTS TO THE ANTIMETABOLITE 6-MERCAPTOPURINE

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Various types of leukemic diseases have been treated in our clinic with different methods of therapy. The therapeutic regimen has varied according to the disease, and has included cortisone, ACTH, urethane, triethylene-melamine, nitrogen mustard, radioactive phosphorus, X ray, and the folic acid antagonists. Our observations on the folic acid antagonists have been previously reported.<sup>1, 2, 3</sup> Leukemic subjects who were in a state of exacerbation of their disease and had received previous therapy were treated with the antimetabolite 6-mercaptopurine (6-MP). Twenty leukemic subjects were observed, including the following types: acute lymphocytic, 10; acute myelocytic, 2; acute monocytic, 3; and chronic myelocytic, 5. Eleven had previously responded well to other types of therapy.

The antimetabolite 6-mercaptopurine is an analogue of the nucleic acid constituent adenine, and the physiologic purine base hypoxanthine. It is one of the large series of such analogues studied by Hitchings and his colleagues<sup>4, 5, 6, 7, 8</sup> and tested for possible chemotherapeutic activity, on the basis that interference with nucleic acid biosynthesis would be more damaging to abnormal than to normal tissues. Recently Burchenal and his co-workers<sup>9</sup> published their account of the clinical evaluation of 6-mercaptopurine in the treatment of leukemia and allied diseases. Their review of the basic experimental observations of the various investigators is very complete. Good clinical and hematologic results were observed in acute leukemia of childhood, occasionally in acute leukemia in adults and, in a few cases, a temporary remission in early and late stages of chronic myelocytic leukemia was produced. 6-MP was also effective in some children who were resistant to folic acid antagonists, to ACTH, and to cortisone.

In the present study, the results were divided into the following groups: good, partial, and failure. A good response was a remission in which all pathological clinical and physical signs disappeared and the peripheral blood picture, including platelets, became entirely normal. A partial response was considered to be improvement in these factors, but with definite residual evidence of leukemia. The term failure is self-explanatory. Bone marrow studies were done, but are not complete enough to include in this report.

## *Dosage of 6-MP*

The antimetabolite was administered orally as 50 mgm. scored tablets. The usual initial dose was approximately 2.5 mgm./kg. (1 mgm. per pound) body weight per day. The dosage was calculated to the closest multiple of 25 mgm. The leukocyte and platelet levels (Rees-Ecker method) were watched closely, and the drug discontinued if these formed elements decreased. When



an optimum level was obtained, a lower maintenance dose was then given. A study of the graphic illustrations of individual cases will reveal the method in decreasing the amount of 6-MP and establishing a maintenance dose.

### *Acute Lymphocytic Leukemia*

A total of 10 individuals who had been previously treated by various forms of therapy were administered 6-MP. The pertinent data are summarized in TABLE 1. The age range was from 2 to 44 years. Previous therapy had consisted of Aminopterin, A-Methopterin, cortisone, and ACTH. A clinical and hematologic remission had occurred in seven subjects prior to an exacerbation of their leukemic disease. A remission was produced with 6-MP in three individuals.

The hematologic data in the three children responding to 6-MP is illustrated graphically in FIGURES 1, 2, and 3. The remission of M. M. (FIGURE 1) was of short duration. The remission of M. K. M. (FIGURE 2) was of longer duration and the child was still alive and doing well  $4\frac{1}{2}$  months after 6-MP was started. The child C. R. (FIGURE 3) had a remission after a marked leukopenia. Aminopterin was then given. 6-MP was again administered.

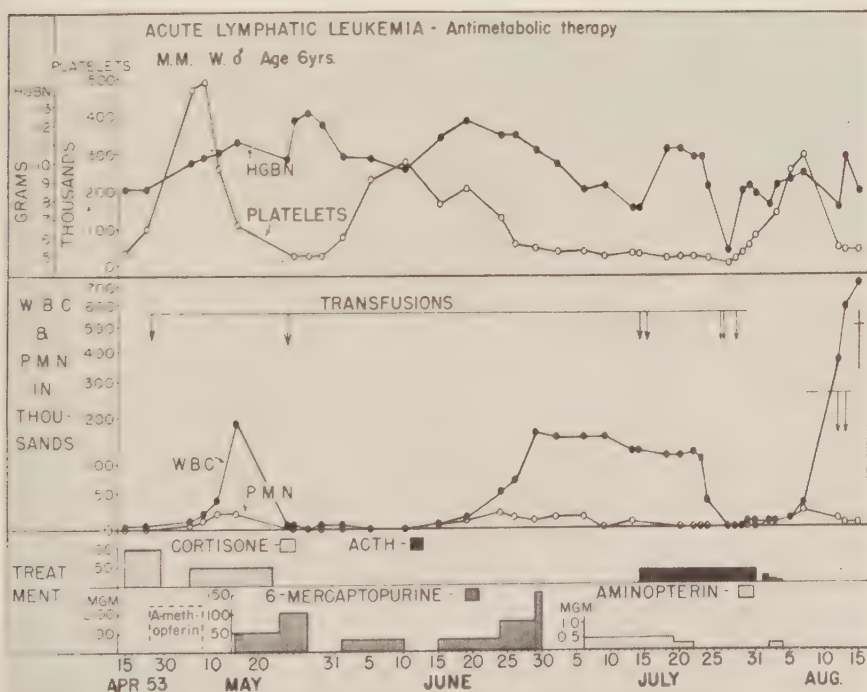


FIGURE 1. Graphic illustration of data in a child having acute lymphatic leukemia. The initial remission followed therapy with cortisone and A-Methopterin. Another remission was obtained with 6-MP. An exacerbation again occurred that responded briefly to Aminopterin. Three distinct remissions occurred in this patient.



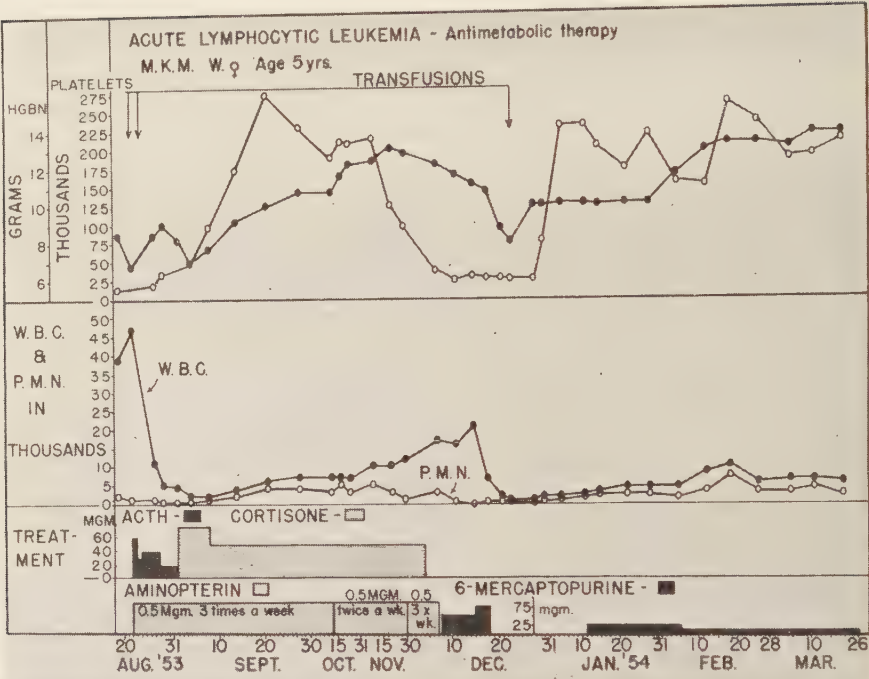


FIGURE 2. Graphic illustration of data in a child having acute lymphatic leukemia. An excellent remission was produced with cortisone and Aminopterin. An exacerbation occurred that responded clinically and hematologically to 6-MP. This patient was alive and in remission as of April 25, 1954. In top frame, platelets are indicated by open circles, and hemoglobin by black circles.

Acute Monocytic Leukemia

Three subjects having acute monocytic leukemia were observed. The pertinent data and previous therapy are tabulated in TABLES 1 and 2. A partial beneficial response was noted in two patients.

The most interesting case was one of pregnancy complicated by leukemia. The data of this patient are graphically illustrated in FIGURE 4. Only cortisone was given before the birth of the child. At no time was the disease completely controlled. The baby boy survived and has developed normally. Following delivery, the mother's condition became critical for several weeks, during which time she was in a continuous state of hemorrhagic diathesis. Therapy with 6-MP was instituted, and a prompt and dramatic decrease in the leukocytes occurred. A marked clinical improvement followed, and the patient was discharged from the hospital and has been observed as an out-patient. She has been in excellent clinical condition, cares for her child, and does part of her housework. Only a partial but beneficial response has been noted. The spleen has continued to be enlarged and annoying to the patient. A maintenance dose has been difficult to establish.

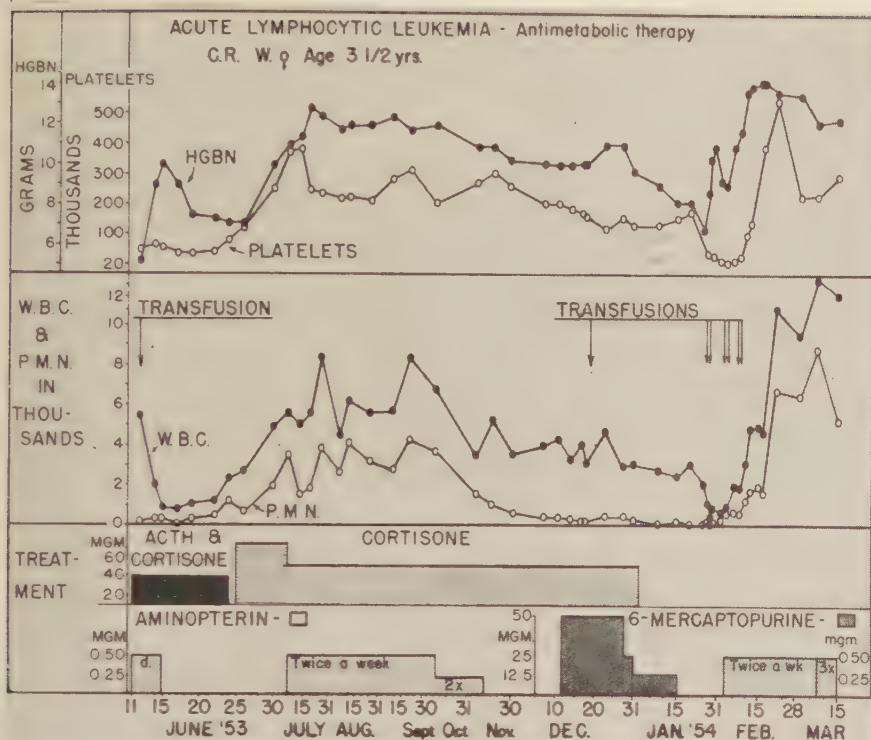


FIGURE 3. Graphic illustration of data in a child having acute lymphatic leukemia. An excellent remission occurred under cortisone and Aminopterin. A severe leukopenia (600 per cubic mm.) followed 6-MP administration, followed by a remission. Aminopterin was then given. With a recent exacerbation (not charted) the child has been started again on 6-MP, and it looks as though another remission will occur. She was alive as of April 25, 1954.

### Acute Myelocytic Leukemia

Two patients having this disease were observed. The pertinent data are summarized in TABLES 1 and 2. No beneficial effect was noted in either instance.

### Chronic Myelocytic Leukemia

Observations were made on five patients having chronic myelocytic leukemia. One was in a late myeloblastic phase. The pertinent data including previous therapy are given in TABLES 1 and 2. In four subjects, a partial beneficial response was obtained. Two patients are of particular interest, the one having chronic myelocytic leukemia and marked eosinophilia, and the other, gout.

The data on the patient with eosinophilia are graphically illustrated in FIGURE 5. Her previous response to X-ray therapy and  $P^{32}$  had been excellent.

TABLE 2

SUMMARY OF 20 CASES OF VARIOUS KINDS OF LEUKEMIA  
The type and results of previous therapeutic agents and 6-mercaptopurine are summarized.

Diagnosis	No.	Type of prior therapy	Prior therapy			6-Mercaptopurine		
			Good	Partial	Fail-ure	Good	Partial	Fail-ure
Acute lymphocytic	10	Aminopterin, A-Methopterin, corti- sone, ACTH	7	1	2	3	0	7
Acute monocytic	3	Aminopterin, TEM, cortisone	0	1	2	0	2	1
Acute myelocytic	2	Aminopterin, urethane, ACTH	0	0	2	0	0	2
Chronic myelocytic	3	P <sup>32</sup> , X ray, TEM, ure- thane	2	0	1	0	3	0
Chronic myelocytic (eosinophilia)	1	ACTH, P <sup>32</sup> , X ray	1	0	0	0	1	0
Chronic myelocytic (blast stage)	1	urethane, X ray, P <sup>32</sup>	1	0	0	0	1	0
Total . . . . .	20		11	2	7	3	7	10

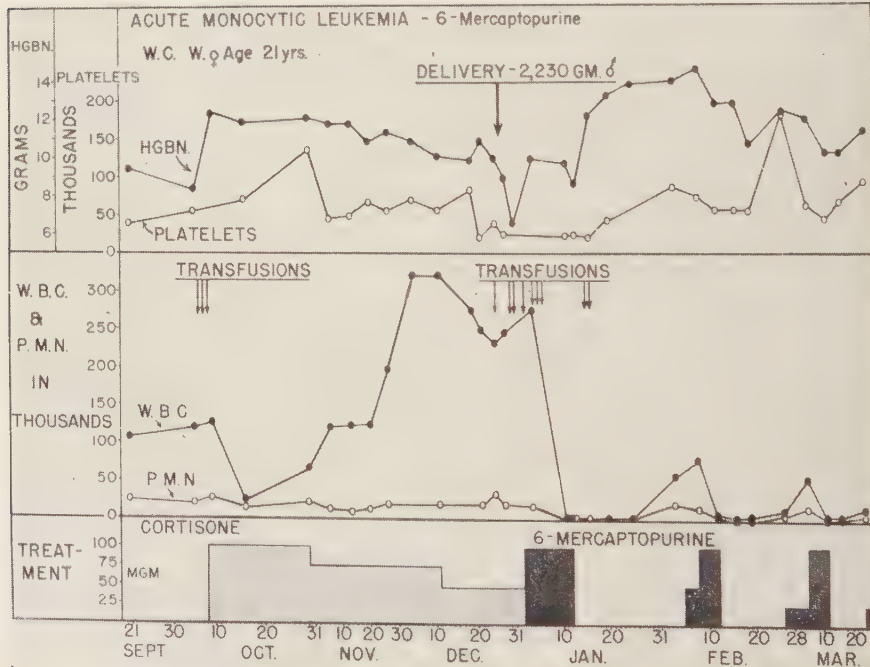


FIGURE 4. This patient had acute monocytic leukemia complicated by pregnancy. Following childbirth, a severe hemorrhagic diathesis occurred. Therapy with 6-MP was started and a severe leukopenia resulted (400 per cubic mm.). Marked clinical improvement followed, and the patient has been able to do housework and care for her child. Maintenance therapy has been difficult to evaluate as to dosage. The spleen continues to be enlarged. This patient was alive and doing very well both clinically and hematologically as of April 25, 1954.

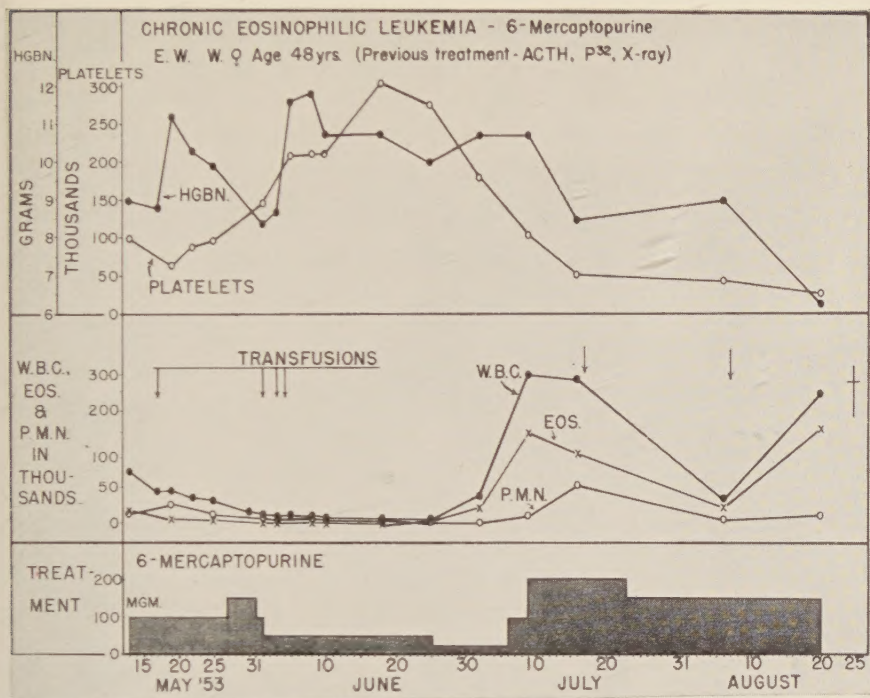


FIGURE 5. Patient afflicted with chronic myelocytic (eosinophilic type) leukemia. Response to previous therapy had been excellent. A temporary improvement occurred, followed by an exacerbation of the leukemic disease and death.

The eosinophilia could not be changed with ACTH, this type of therapy being tried before irradiation was given. After 6-MP was administered, the patient gained weight. The tremendous spleen decreased markedly in size but was still palpable. An exacerbation then occurred, and the patient expired.

The elderly male having chronic myelocytic leukemia and gout was given 6-MP (FIGURE 6). No other therapy was used that might prevent an acute gouty episode, so frequent when such patients are actively treated for their leukemic disease. All residual evidence of the gout (present in the feet only) disappeared. To use the patient's own words, "My feet are entirely normal and better than they have been for a year and a half." The uric acid levels remained elevated during the entire period of observation. The results at present are considered partial, as the hematologic picture is not normal, and the spleen and liver are still enlarged. The antimetabolite is being continued.

The patient having a terminal myeloblastic phase of chronic myelocytic leukemia has partially responded to six weeks of treatment. The leukocyte level has decreased to normal, and the mature neutrophils and platelets have increased in absolute numbers. The bone marrow shows little change. She looks and feels much better, and the spleen has decreased in size. She has gained six pounds in weight. The therapy is being continued.



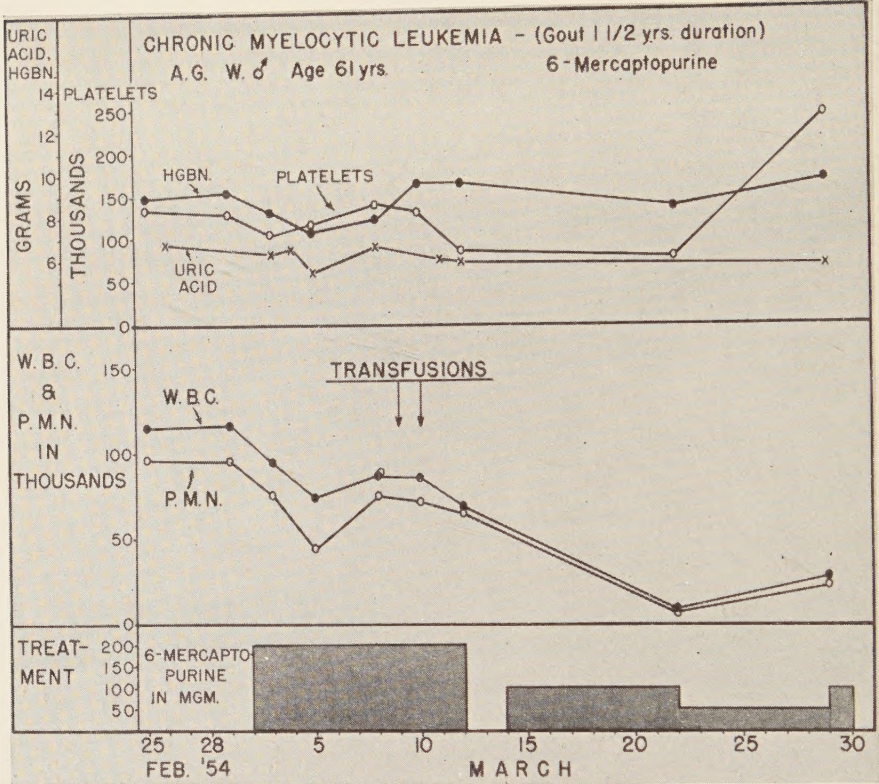


FIGURE 6. Graphic illustration of a patient having chronic myelocytic leukemia and gouty arthritis. No acute gouty episode occurred with 6-MP therapy, his feet being better than during the previous one and one half years. The spleen remains enlarged. Therapy has been continued and the patient was doing well as of April 25, 1954. Uric acid in mgm. per cent.

*Toxic Effects*

A dramatic rapid leukopenia was observed in some patients. A thrombocytopenia developed at times. It is very difficult to differentiate between the possible toxic effects of 6-MP and the naturally occurring events in terminal leukemia. Of course, this confusion makes therapy difficult, particularly in judging dosage. An elevated temperature occurred in some patients.

No oral lesions or gastrointestinal symptoms were observed. Particular caution was exercised in stopping the drug when the leukocyte level decreased, which may account for the lack of toxic effects in our series.

*Discussion*

It seems apparent that 6-mercaptopurine may be beneficial in some individuals who have previously responded to other types of therapy. Good results may occur in some individuals who have not previously responded. 6-MP is somewhat different from the folic acid antagonists in that some individuals having chronic myelocytic leukemia may respond beneficially. The study of

the patient afflicted with gout is of considerable interest, and it brings forth the question as to whether or not such a powerful antimetabolite might be tried in small doses in patients afflicted with uncomplicated gout. Usually there is an exacerbation of gout in patients having hematologic diseases during periods of active therapy. Hyperuricemia is frequently observed in leukemic patients.

It has been our experience that the clinical evaluation of any new antimetabolite requires considerable time and patience. Only careful observations will reveal proper dosage, beneficial results, and toxic manifestations.

### Conclusions

(1) The antimetabolite 6-mercaptopurine was administered to 20 leukemic patients who had been treated previously with other methods of therapy. The following types of leukemia were observed: acute lymphocytic, 10; acute monocytic, 3; acute myelocytic, 2; and chronic myelocytic, 5.

(2) A remission occurred in three patients having acute lymphocytic leukemia who had previously responded to steroids and folic acid antagonists. A partial beneficial response occurred in two acute monocytic leukemias and in five chronic myelocytic leukemias.

(3) Unusual leukemic subjects treated with 6-mercaptopurine included a case of pregnancy complicated with acute monocytic leukemia, one case of gout and chronic myelocytic leukemia, and a case of chronic myelocytic leukemia with marked eosinophilia. All responded favorably to the antimetabolite.

(4) Adverse effects consisted of leukopenia, thrombocytopenia, and an elevated temperature. Possible toxic effects, however, were difficult to differentiate from the usual terminal events of leukemic diseases.

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